Towards a best practice for Campylobacter prevention at farm and house level

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Welcome to CHRO 2013

The University of Aberdeen warmly welcome delegates and invited guests to the 17th International Workshop on Campylobacter, Helicobacter and Related Organisms. This conference returns to Europe for the first time in six years.

The CHRO Conference 2013 will be the leading international conference, where cutting edge research meets medicine, industry and policy around the topics of this important group of organisms. This will be a unique opportunity to see the recent ground-breaking developments with these important pathogens at this pivotal time in our knowledge and understanding. The coming together of genomics, epidemiology and basic and systems biology with applied biology and health have underpinned the philosophy of this Conference leading to a number of Themes that thread their way through the proceedings:

What have we learnt from 30 years of CHRO research?

Which CHRO organisms will be of biggest global concern in 10 years time?

How do we minimise the disease burden of CHRO?

How will omics and systems biology transform CHRO research and understanding?

The organising committee would like to thank all of our Sponsors both here in the UK and internationally for their generous support. Thanks also to our advisors at home and abroad, to the plenary speakers and the session chairs for their time and extensive expertise and advice. The organising committee would like to thank our Event Organisers CPD Services of University of Aberdeen, for their continual support and assistance.

Welcome!

Ken Forbes
Emad El Omar
Norval Strachan
Georgina Hold

Disclaimer

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Campylobacter, Helicobacter, and Related Organisms
CHRO conference

15–19 September 2013

AECC, Aberdeen

PROGRAMME

Sunday 15th September 2013

14.00 – 17.00 Registration

17.00 – 17.30 **Welcome:** Professor Ian Diamond
Opening Remarks: Ken Forbes, Norval Strachan, Georgina Hold and Emad El-Omar

17.30 – 18.30 **Plenary Talks**
1. Arie Havelaar Campylobacter: View from the EFSA BIOHAZ panel and the Netherlands
2. Emad El-Omar Helicobacter – Helicobacter pylori infection: past, present and future

18.30 – 20.00 **Welcome Reception, AECC**

Monday 16th September 2013

08.15 – 08.30 **Opening remarks**

08.30 – 10.00 **Plenary Session 1**
What have been the main paradigm shifts in CHRO research in the last decade?

Session Chairs	Francis Mégraud	Ken Forbes
08.30–09.00	**3. Diane Newell**	Campylobacter jejuni - the answer is 42 but what are the questions?
09.00–09.30	**4. John Atherton**	Developments in the understanding of Helicobacter virulence – a 10 year perspective
09.30–10.00	**5. Nigel French**	10 years of effort to tackle human campylobacteriosis in New Zealand

10.00 – 10.30 *Refreshment break*

10.30 – 12.30 **Parallel Session 1**

Session Theme	Helicobacter pathogenesis from the host perspective	Campylobacter pathogenesis from the host perspective
Session Chairs	Mona Bajaj-Elliott and Indrani Mukhopadhya	Paul Everest and Paul Wigley
10.30–10.50	**6a. Natalia Castano Rodriguez** The role of autophagy and inflammasomes in Helicobacter pylori-related gastric cancer	**6b. Roland Buecker** Diarrheal mechanisms in *Campylobacter jejuni* enteritis
10.50–11.10	**7a. Richard Ingram** The human IL-17/Th17 response to Helicobacter pylori infection	**7b. Lieneke Bouwman** Inflammasome activation by *C. jejuni*
11.10–11.25	**8a. Anna Roujeinikova** Molecular mimicry between *Helicobacter pylori* CagA and host proteins: implications for pathogenesis.	**8b. Martin Stahl** SIGIRR-deficient mice, a novel infection model for the study of innate immune responses to *Campylobacter jejuni*
11.25–11.40	**9a. Sabine Kienesberger** Effects of Helicobacter pylori on host immunity and gut microbiome using the C57/Bl6 mouse model.	**9b. Astrid Heikema** A role for Siglecs in the recognition of Guillain-Barré syndrome-related *Campylobacter jejuni* strains
11.40–11.55	**10a. Mark Whary** Gastric colonization with a restricted commensal flora replicates the promotion of neoplastic lesions by diverse	**10b. Shadi Zakai** *Campylobacter jejuni* at the host pathogen interface: The role of periplasmic chaperones in the biogenesis of outer membrane proteins
11.55–12.10	**11a. Eleonora Altman** The Potential of Dextran-Based Glycoconjugates for Development of Helicobacter pylori Vaccine	**11b. Markus M. Heimesaat** *Campylobacter jejuni* induces acute non-self-limiting enterocolitis in gnotobiotic IL-10$^{-/-}$ mice via Toll-like-receptor-2 and -4 signaling
12.10–12.25	**12a. Lydia Wroblewski** Use of a novel ex vivo three-dimensional system to define host-microbial interactions with carcinogenic potential	**12b. Monika Keelan** Meaningful Dissemination of Community-Driven *H. pylori* Microbiology Research in Indigenous Arctic Communities
12.25–12.30	Summary	Summary

12.30 – 13.30 Lunch

12.45 – 13.45 **Lunchtime networking session 1:**

13.00 – 14.15 **Poster session 1**

14.30 – 16.30 **Parallel Session 2**

Session Themes	**Treatment / therapeutics and sequelae**	**Pathogenesis from the bacterial perspective**
Session Chairs	John Thomson and Mirko Rossi	Billy Bourke and Nicola Jones
14.30–14.50	**13a. Stefan Bereswill** *Campylobacter jejuni* infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses	**13b. Julie Ann Naughton** Sticky and Sweet: The Interaction of *Campylobacter jejuni* and *Helicobacter pylori* with purified mucins
14.50-15.10	**14a. Sebastian Suerbaum** Intestinal microbiota composition of interleukin-10 deficient C57BL/6J mice and susceptibility to *Helicobacter hepaticus*-induced colitis	**14b. Jennifer Noto** CagA-dependent downregulation of microRNA-320 by carcinogenic *Helicobacter pylori* promotes expression of the cell survival protein, Mcl-1 *in vitro* and *in vivo*
15.10–15.25	**15a. Richard Hansen** A comprehensive overview of *Campylobacter* and *Helicobacter* in de-novo Paediatric Inflammatory Bowel Disease.	**15b. Armelle Ménard** The cytolethal distending toxin of *Helicobacter pullorum* targets vinculin and cortactin, and triggers formation of lamellipodia in intestinal epithelial cells
15.25–15.40	**16a. Astrid Heikema** Particular lipooligosaccharide loci and capsule types co-occur in Guillain-Barré syndrome-associated *Campylobacter jejuni* strains	**16b. Christine Josenhans** *H. pylori* CagN, a protein which targets human ubiquitin and related small modifiers
15.40–15.55	**17a. Hans Linde Nielsen** A randomized, double-blind, placebo-controlled trial of azithromycin in *Campylobacter concisus* positive patients with diarrhoea	**17b. Markus M. Heimesaat** Intestinal microbiota shifts towards elevated commensal Escherichia coli loads abrogate colonization resistance against Campylobacter jejuni in mice
15.55–16.10	**18a. Jun Lin** Critical role of a putative lytic transglycosylase in β-lactam resistance in *Campylobacter jejuni*	**18b. Giovanni Suarez** PgdA deacetylase regulates evasion of host defense mechanisms by *Helicobacter pylori* that contribute to bacterial persistence
16.10–16.25	**19a. Sean Pendleton** Impact of Rearing Conditions on Arsenic Resistance in *Campylobacter spp.*	**19b. Mona Bajaj-Elliott** Glycosylated moieties' of *Campylobacter jejuni* flagella modulate Dendritic Cell IL-10 expression *via* Siglec-10 receptor engagement
16.25–16.30	Summary	Summary

16.30 – 17.45 **Poster Session 2 and Coffee**

18.30 – 20.30 **Slow Food**

Tuesday 17th September 2013

08.30 – 10.00 **Plenary Session 2**
Which CHRO organisms will be of greatest concern in the next ten years?

Session Chairs	Emad El-Omar	Christine Szemansky & Erin Gaynor (TBC)
08.30–09.00	**20. Hazel Mitchell**	*Helicobacter species* of concern: Crystal ball predictions for the next decade.
09.00–09.30	**21. Rob Mandrell**	Campylobacter
09.30–10.00	**CHRO 2015 Presentations**	

10.00 – 10.30 *Refreshment break*

10.30 – 12.30 **Parallel Session 3**

Session Themes	**Epidemiology – Helicobacters**	**Clinical epidemiology of Campylobacter**
Session Chairs	Guillermo Perez Perez and Emad el-Omar	Gordon Nichols and Pascal Michel
10.30–10.50	**22a. María José Figueras** What have we learnt of the genus *Arcobacter* since its description in 1991?	**22b. Arnoud van Vliet** Rapid, alignment-free analysis of whole genome sequences of *Campylobacter jejuni* and *Campylobacter coli* for molecular epidemiology
10.50–11.10	**23a. Guillermo Perez** Improved methodology for the primary culture of *Helicobacter pylori* from gastric biopsies.	**23b. Martijn Bouwknegt** Recent increase in campylobacteriosis incidence in The Netherlands potentially related to proton-pump inhibitor use
11.10–11.25	**24a. Jie Liu** Gene-*specific* PCR analysis of *Helicobacter suis* in China	**24b. Laura MacRitchie** Estimating the Financial Burden and Disease Severity of *Campylobacter* in Scotland
11.25–11.40	**25a. Monika Keelan** *Helicobacter pylori* Infection in the Yukon Territory of Canada	**25b. Julie Arsenault** The seasonality of campylobacteriosis: are we missing something?
11.40–11.55	**26a. Lien De Cooman** Occurrence of *Helicobacter suis* DNA on porcine slaughterhouse carcasses.	**26b. Monika Koziel** *Campylobacter ureolyticus*: an Emerging Gastrointestinal Pathogen?
11.55–12.10	**27a. Anne-Marie Van den Abeele** *Arcobacter* in Humans: Intestinal Colonizer or Pathogen?	**27b. Alison Cody** Genomic surveillance of human *Campylobacter* isolates obtained over a one year period, from Oxfordshire, UK.
12.10–12.25	**28a. Arnoud van Vliet** Whole genome-based phylogenetic clustering of *Helicobacter pylori* correlates with geographic origin and virulence factor-based typing schemes	**28b. Bettina Rosner** Description of the epidemiological pattern of campylobacteriosis in Germany from 2001–2010
12.25–12.30	Summary	Summary

12.30 – 13.30 *Lunch*

12.45 – 13.45 **Lunchtime networking session 2:**

13.00 – 14.15 **Poster Session 3**

14.30 – 15.55 **Parallel Session 4**

Session Themes	**Biology and Genomics of CHRO 1**	**Campylobacter interventions**
Session Chairs	David Kelly and Christine Josenhans	Ian Connerton and Jaap Wagenaar
14.30–14.50	**29a. Cynthia M. Sharma** Regulatory RNAs in the pathogenic Epsilonproteobacteria *Helicobacter pylori* and *Campylobacter jejuni*	**29b. Peter van der Logt** *Campylobacter* in broilers: Risk assessment as a basis for selecting a performance target
14.50–15.05	**30a. Michael Taveirne** Mapping the *in vivo* transcriptome of *Campylobacter jejuni* using RNAseq	**30b. Arno Swart** Microbiological criteria as a decision tool for controlling Campylobacter on broiler meat
15.05–15.20	**31a. Eduardo Taboada** CIST: the Campylobacter *In Silico* Typing server, a resource for integrated comparative genomic analysis of *Campylobacter jejuni*	**31b. Maarten Nauta** Risk-Based Microbiological Criteria: A Tool To Control *Campylobacter*
15.20–15.35	**32a. Craig Parker** The complete genome sequences of 65 *Campylobacter jejuni* and *C. coli* strains	**32b. Mogens Madsen** Towards a best practice for *Campylobacter* prevention at farm and house level
15.35–15.50	**33a. Jane Mikhail** Comparative gene-by-gene analyses of Helicobacter pylori genomes	**33b. Mike Hutchison** Monitoring of campylobacters in UK poultry slaughter batches and carcasses and collection of information from primary production and processing for risk factor elucidation
15.50–15.55	Summary	Summary

15.55 – 16.30 *Refreshment break*

16.30 – 18.00 **Parallel Session 5**

Session Themes	**Biology and Genomics of CHRO 2**	**Campylobacter vaccines**
Session Chairs	David Kelly and Christine Josenhans	Ian Connerton and Jaap Wagenaar
16.30–16.50	**34a. Erin Gaynor** *Campylobacter jejuni* peptidoglycan-modifying enzymes: new players controlling helical shape and pathogenic properties	**34b. Harald Nothaft** The *Campylobacter jejuni* protein glycosylation pathway-derived heptasaccharide is an effective chicken vaccine
16.50–17.05	**35a. Mark Reuter** Defining the role and regulon of the *C. jejuni* peroxide stress regulator (PerR)	**35b. Olena Redkyna** The creation of an anti-*Campylobacter jejuni* multivalent vaccine for humans.
17.05–17.20	**36a. Arthur Hinton Jr** Enhancing Aerobic Growth of Campylobacter in Media Supplemented with Organic Acids	**36b. Katarzyna Radomska** Immunogenicity of a *Campylobacter jejuni* flagellin-based subunit vaccine in chickens
17.20–17.35	**37a. Jamie Luo** Modelling Bacterial Persistence in Campylobacter jejuni.	**37b. Alexandra Armstrong** Use of a Recombinant Attenuated *Salmonella* Typhimurium Vaccine Vector for the Reduction of *Campylobacter jejuni* in Broiler Chickens

Session Themes	Biology and Genomics of CHRO 2	Campylobacter vaccines
Session Chairs	David Kelly and Christine Josenhans	Ian Connerton and Jaap Wagenaar
17.35–17.50	**38a. Bruce Pearson** Two small non-coding RNAs post-transcriptionally control flagellar gene expression in *Campylobacter jejuni*	**38b. Brenda Allen** Development of an adenovirus vectored vaccine for the prevention of colonization of poultry by *Campylobacter*
17.50–18.00	Summary	Summary

18.30 – 19.30 **Traditional Campylobacter vs. Helicobacter soccer match**

19.30 **Free evening**

Wednesday 18th September 2013

08.30 – 09.30 **Plenary Session 3**
How do we minimise the disease burden?

Session Chairs	Norval Strachan	Stephen Trent
08.30–09.00	**39. Rick Peek**	New Insights into the pathogenesis of *H.pylori* infection
09.00–09.30	**40. Charles Milne**	The FSA'S Campylobacter Reduction Strategy
09.30–10.00	**41. Francis Mégraud**	Prevention of gastric cancer by eradication of *H. pylori*. Is it time for mass eradication?

10.00 – 10.30 *Refreshment break*

10.30 – 12.00 **Parallel Session 6**

Session Themes	(Rapid) methods for detection of CHRO	Non-poultry epidemiology and ecology of Campylobacter
Session Chairs	Bob Madden and Richard Hansen	Jonas Waldenstrom and Philip Carter
10.30–10.50	**42a. Angela Cornelius** MBiT: Molecular typing of *Campylobacter jejuni* and *C. coli* in less than six hours and under €10!	**42b. Jonas Waldenström** The call of the wild - lessons from environmental campylobacters
10.50–11.10	**43a. Gereon Göttner** A novel Immuno-line Assay enables highly specific and sensitive serologic diagnosis of *H. pylori* Infection and predicts histopathologic progression	**43b. Christian Penny** Real-time surveillance of *campylobacter* linked to detection in environmental waters and wastewater
11.10–11.25	**44a. Andrew Pridmore** Optimization of atmospheric gas mixtures for cultivation of *Campylobacter jejuni* and *C. coli*	**44b. Lapo Mughini Gras** Combining source attribution and epidemiological data: a tool for investigating source-associated risk factors for human campylobacteriosis
11.25–11.40	**45a. Krunoslav Bojanic** Comparison of six culture protocols for isolation of *Campylobacter* spp. from faecal and meat samples	**45b. Maarten Gilbert** Prevalence, host association, and diversity of *Campylobacter*, *Arcobacter*, and *Helicobacter* in reptiles and amphibians

Session Themes	(Rapid) methods for detection of CHRO	Non-poultry epidemiology and ecology of Campylobacter
Session Chairs	Bob Madden and Richard Hansen	Jonas Waldenstrom and Philip Carter
11.40–11.55	**46a. Michael Rothrock** Optimization and validation of a *Campylobacter* genera-specific qPCR assay: A molecular tool to test anecdotal *Campylobacter* species prevalence within environmental samples	**46b. Annette Nygaard Jensen** Quantitative estimation of *Campylobacter jejuni* survival in house flies at 20°C and 42°C after inoculation with 3×10^3 CFU.
11.55–12.10	**47a. Kerstin Stingl** Crucial parameters for a reliable quantification of viable *Campylobacter* by real-time PCR	**47b. Benjamin Hetman** Enrichment-based Isolation of *C. jejuni* from Environmental Samples resulting in Biased Genetic Diversity
12.10–12.25	**48a. Melissa Jansen van Rensburg** A Genomic Approach to the Evaluation of a Real-Time PCR Assay for Speciation of *Campylobacter jejuni* and *Campylobacter coli.*	**48b. Ben Pascoe** Biofilm and its impact on the biology of *Campylobacter*
12.25–12.30	Summary	Summary

12.00 – 13.00 *Lunch*

13.00 – 14.25 **Parallel Session 7**

Session Themes	Campylobacter control: practical interventions	CHRO taxonomy open meeting
Session Chairs	Chris Bayliss and Declan Bolton	Stephen On
13.00 – 13.20	**49a. Ian Connerton** The bacteriophage carrier state of *Campylobacter jejuni*	
13.20 – 13.35	**50a. Dean Burfoot** Efficacy of Selected Intervention Methods to Reduce Campylobacter Contamination on Chicken Carcasses in UK Slaughterhouses	
13.35 – 13.50	**51a. Lisa Williams** Polyunsaturated Fatty Acid Diets alter the immune response of chickens to *Campylobacter*	
13.50 – 14.05	**52a. Sigurborg Daðadóttir** 2008-2012 fly screening ventilation inlets of broiler houses on high risk farms in Iceland to reduce flyborne transmission of Campylobacter: Impact on flock prevalence and public health.	
14.05 – 14.20	**53a. Hanieh Mousavian** Combined steam and ultrasound treatment of broilers at slaughter - a promising intervention to significantly reduce numbers of campylobacters on carcasses and improve food safety	
14.20 – 14.25	Summary	

14.25 – 14.55 *Refreshment break*

14.55 – 16.55 **Parallel Session 8**

Session Themes	**CHRO omics**	**Poultry epidemiology and ecology of Campylobacter**
Session Chairs	Sam Sheppard and Bill Miller	Merete Hofshagen and Nick Sparks
14.55 – 15.15	**54a. David J Kelly** A molecular explanation for microaerophily in *Campylobacter jejuni*	**54b. Hanne Rosenquist** CamCon - novel approaches to control *Campylobacter* in primary poultry production
15.15 – 15.35	**55a. Ian Connerton** Transcriptome analysis of *Campylobacter* in response to bacteriophage infection	**55b. Gemma Chaloner** A comparison of *Campylobacter* infection in commercial broilers under different management systems; preliminary results.
15.35 – 15.50	**56a. Arnoud van Vliet** Characterising CHRO transcriptomes: an RNA-seq led treasure hunt	**56b. Djamila Moulay** A mechanical hypothesis for the lag phase of Broiler colonization with *Camplyobacter jejuni*
15.50 – 16.05	**57a. Juliane Krebes** Comprehensive methylome analysis of the human gastric pathogen, *Helicobacter pylori*	**57b. Marta Cerdà-Cuéllar** House fly (*Musca domestica*) as a vector for *Campylobacter jejuni* and *Campylobacter coli* in Spanish broiler farms
16.05 – 16.20	**58a. Sabine Kienesberger** Horizontally acquired genetic elements drive genome evolution, virulence, and niche specificity in the pathogen *Campylobacter fetus.*	**58b. Tomasz Seliwiorstow** The Role of Slaughter Practices in the Transfer of *Campylobacter* Contamination between Batches
16.20 – 16.35	**59a. Guillaume Meric** A genome-wide association study of *Campylobacter* survival in the poultry processing chain	**59b. Ovidiu Rotariu** An integrated model to estimate the source of *Campylobacter* infection in broiler houses
16.35 – 16.50	**60a. Patrick Biggs** The pathogenomics of *Campylobacter jejuni* in New Zealand	**60b. Katell Rivoal** A large scale survey describing the relationship between broilers and human campylobacteriosis
16.50 – 16.55	Summary	Summary

19.00 for 19.30 **Conference dinner**

Thursday 19th September 2013

08.30 – 10.00 **Plenary Session 4**

How will omics and systems biology transform CHRO research and understanding

Session Chairs	Craig Parker	Georgina Hold
08.30–09.00	**61. Brendan Wren**	What has the Campylobacter jejuni genome done for us?
09.00–09.30	**62. Sebastian Suerbaum**	Genome variation in Helicobacter pylori
09.30–10.00	**63. Martin Maiden**	From MLST to genomics: the gene-by-gene approach to population annotation for *Campylobacter*.

10.00 – 10.30 *Refreshment break*

10.30 – 12.30 **Parallel Session 9**

Session Themes	**Evolution: diversity, emergence and speciation**	**Virulence and biofilms**
Session Chairs	Chris Bayliss and Declan Bolton	James Fox and Nadeem Kaakoush
10.30–10.50	**64a. Lea Lango-Scholey** Investigation of the alterations in the phase variable genes of Campylobacter jejuni 11168 during colonisation of chickens	**64b. Derrick Samuelson** The novel *Campylobacter jejuni* effector protein CiaD induces mitogen-activated protein kinase signaling pathways required for host IL-8 secretion
10.50–11.10	**65a. Barbara Binney** Phenotype variation of *Campylobacter jejuni* sequence types associated with wild birds and ruminants	**65b. Terry Kwok** Novel mechanisms of IL-8 induction by CagL of the *Helicobacter pylori* type IV secretion apparatus
11.10–11.25	**66a. Rauni Kivistö** Genomic diversity among five *Campylobacter jejuni* chicken isolates representing the ST-677 clonal complex	**66b. Nadeem Kaakoush** Pathogen comparative genomics and host transcriptomics to investigate the pathogenic potential of Campylobacter concisus
11.25–11.40	67a. María Domínguez-Bello *H. pylori* strain dominance via transformation	67b. Elaine Allan Induction of exopolymeric matrix in response to a host-specific signal in Campylobacter jejuni
11.40–11.55	**68a. Koji Yahara** Chromosome painting *in silico* in a bacterial species reveals fine population structure	**68b. Roberto Barrozo** Modulation of the Helicobacter pylori Type IV Secretion System Function in Response to Adaptive Immune Pressure
11.55–12.10	**69a. Sabine Kienesberger** Comparative genome analysis identified horizontally acquired genes important for virulence and niche adaptation in *Campylobacter fetus* and *Campylobacter curvus*.	**69b. Paul Plummer** RNA-seq of Campylobacter jejuni using a novel in vivo bile model
12.10–12.25	70a. Jens Andre Hammerl Genome sequence of Campylobacter phage CP21	70b. Bow Ho A study of formation, carbohydrate composition and mechanical forces exhibited by *Helicobacter pylori* biofilm
12.25–12.30	Summary	Summary

12.30 – 13.00 **Award of Student Prizes, closing remarks and passing of the CHRO Torch**

13.00 **Grab and Go Lunch**

Orals

O1 - Advances in risk assessment of campylobacteriosis and their relevance for risk management

Arie Havelaar[1,2]
[1]Utrecht University, Utrecht, The Netherlands, [2]National Institute for Public Health and the Environment, Bilthoven, The Netherlands

Since the first published risk assessment of Campylobacter on chicken meat in 1999, many studies from different European countries and North America have followed. All follow the same structure, are based on limited datasets and come to similar key conclusions. Recent work has focused on integrating more detailed data from several steps of the food chain, on implementing advanced modeling tools (in particular Bayesian approaches) and extending the geographical range of risk assessments (e.g. Japan, Senegal and Argentina). Even though chicken meat is generally considered an import exposure pathway, it is by no means the only pathway. Some risk assessment studies have focused on exposure by drinking or swimming water, salad crops and dairy. Several fundamental assumptions in risk assessment studies of Campylobacter have remained unchallenged, yet are not consistent with the epidemiology and pathobiology of campylobacteriosis. Key challenges to develop more realistic risk assessment models include the assumptions that subsequent exposures are independent, that the conditional probability of illness given infection is independent of the exposure dose and that all Campylobacters behave equal in the food chain and in human hosts. Approaches to address these challenges are becoming available. Risk assessment is increasingly used to inform risk management decisions. The European Food Safety Authority has published an evaluation of control options for Campylobacter on chicken meat along the food chain, which has been the basis of an economic evaluation. Current debate focuses on the feasibility, effectiveness and efficiency of microbiological criteria for Campylobacter on chicken meat.

O2 - Helicobacter – *Helicobacter pylori* infection: past, present and future

Emad El-Omar
University of Aberdeen

O3 - *Campylobacter jejuni* - the answer is 42 but what are the questions?

Diane Newell
Foodborne Zoonoses Consultancy Ltd, Andover, UK

According to *The Hitchhiker's Guide to the Galaxy* by Douglas Adams the number 42 is, "The Answer to the Ultimate Question of Life, the Universe, and Everything". Unfortunately no one knows what the question is. For the last 32 years, we have accumulated vast amounts of information about *C. jejuni*, most of which has been presented at one of the 16 previous International Workshops. Nevertheless, when reviewing the abstract books, it seems that the questions addressed to *C. jejuni* over this period have remained essentially the same. For example; how does it cause disease?; where does it come from?; how can we control the disease? and so on. Why, therefore, when so many have put in so much effort, do these questions remain pertinent for the 17th Workshop. Perhaps it is time to rethink our research approaches, Much of the research effort, particularly over the last 10 years, has been technology driven - but has throwing yet another, albeit more modern, technique at the organism added any more information than the older methods? In this review I will attempt to assess the questions consistently addressed and the approaches recently adopted. To this end I have sought the views of other Campylobacter reseachers in an attempt both to broaden my views and to share the blame!

O4 - Developments in the understanding of Helicobacter pylori virulence

John Atherton
Nottingham Digestive Diseases Centre NIHR Biomedical Research Unit, University of Nottingham, Nottingham, UK

Helicobacter pylori and humans have co-evolved throughout their existence. On the human side this has influenced our physiology and immunology. Largely unknown environmental influences on our relationship with H. pylori have led to the epidemics of peptic ulcer disease and gastric adenocarcinoma in the last 150 years. More recently, reductions in H. pylori prevalence in many populations have led to these diseases becoming much less common. However, the absence of H. pylori for the first time in human evolutionary history is a rapid change to which we have not fully adapted: this has led to an increase in acid-related oesophageal disease (including oesophageal adenocarcinoma) and perhaps, controversially, has contributed to the increase in some systemic disorders of modern human life such as asthma and atopy. H. pylori produces host interaction factors to modulate its interaction with humans and help its survival in and transfer between hosts. As the balance of this interaction is towards causing serious disease, they are rightly termed virulence factors. However, only a minority of H. pylori infections result in disease; this is due in some cases to absence of these factors and in others to expression of less interactive forms. The best understood virulence factor is the type IV secretion system encoded on the cag Pathogenicity island. This modulates human epithelial cells through direct signalling following attachment to integrins, through translocating CagA into the cell, and through innate immune recognition. The cag PaI is present in about 70% of strains, but CagA itself is polymorphic and some forms have much more profound effects on epithelial cells and are more closely associated with disease. H. pylori has three other type IV secretion systems, and there is current interest in whether another of these, Tfs4, may also be involved in virulence. H. pylori produces an auto-transported toxin, VacA, which forms membrane pores and induces cell damage. VacA is highly polymorphic and possession of some forms causes more damage to cells and model systems and is tightly associated with disease. Other important polymorphic virulence factors include adhesins and a protein of unknown function termed oipA. Although humans evolve slowly, H. pylori evolves rapidly in the human stomach throughout the lifetime of its host, and this applies particularly to some of its virulence factors. This may be an adaption mechanism to suit a changing niche. The influence of this rapid evolution on pathogenicity is poorly understood, but is likely to be important.

O5 - Tackling the human campylobacteriosis epidemic in New Zealand: a summary of 10-years of effort

Nigel French
Infectious Disease Research Centre, Institute of Veterinary, Animal and Biomedical Sciences, Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, Palmerston North, New Zealand

In 2006 New Zealand witnessed an unprecedented number of campylobacteriosis cases. The slowly building 'epidemic' peaked at 16,000 notified cases over the 12 month period, which was extraordinarily high for a population of just 4 million people. After a concerted effort by regulators and industry to reduce the level of the hazard in the poultry supply, the number of notifications declined by approximately 50% in 2007/8, and this has persisted to the present day. Recent estimates of the associated savings to the New Zealand economy stand at around NZ$70M per annum. As a result of this dramatic reduction, attributed to improvements in one major food source, the epidemiology has changed; notification rates are now higher in rural compared to urban areas, and non-poultry sources, particularly ruminants, are estimated to be responsible for a much higher proportion, though not absolute number, of cases than in previous years. In this presentation, a summary of the key decisions that were made to tackle the epidemic, and the science behind the decision making, will be outlined. This will include a description of the development and application of molecular and modelling tools used to identify the animal reservoirs and pathways of infection, and the techniques used to understand and monitor the epidemiological patterns in space and time. The situation in New Zealand will be compared with other countries, and questions will be addressed concerning the manner in which the improvements in public health were achieved, and how applicable they would be in other settings. In addition, the need to avoid complacency, reduce rates further and apply our advancing knowledge of the evolution of Campylobacter spp. to anticipate and mitigate against the emergence of new strains, will be discussed.

O6a - The role of autophagy and inflammasomes in Helicobacter pylori-related gastric cancer

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Introduction: Autophagy and inflammasomes are ancient innate immune mechanisms that are linked by mutual regulation. Because *Helicobacter pylori*-related gastric cancer (GC) is a progressive process initiated by inflammation, we investigated the role of both autophagy and inflammasome pathways in this pathology. Methods: Fifty-three polymorphisms were detected by PCR, real-time PCR and MALDI-TOF mass spectrometry in 310 ethnic Chinese individuals (87 non-cardia GC cases/223 controls with functional dyspepsia). Gene expression of 168 molecules involved in the autophagy and inflammasome pathways was assessed through quantitative real-time RT-PCR in mammalian cells challenged with *H. pylori*. Results: Five polymorphisms showed significant associations with GC. On multivariate analysis, *CARD8*-rs11672725, *ATG16L1*-rs2241880 and *IGRM*-rs4958847 remained statistically significant. THP-1 cells challenged with two *H. pylori* strains, GC026 (GC) and 26695 (gastritis), showed down-regulation of *NLRP12* and *NLRX1* and up-regulation of *PTGS2*, however, GC026 showed the greatest changes. Despite down-regulation of molecules involved in early stages of the inflammasome pathway, persistent up-regulation of *NF-kB* in *H. pylori* GC026-challenged THP-1 cells was observed. Molecules involved in the induction and maturation of autophagosomes showed significant down-regulation in *H. pylori* GC026-challenged AGS cells. Remarkably, *IGRM*, a gene encoding an IFN-inducible GTPase that stimulates autophagic and inflammasome-related antimicrobial activities, showed decreased expression levels in *H. pylori* GC026-challenged AGS cells. Discussion: Highly virulent *H. pylori* strains may not only evade/exploit autophagy for survival and replication but may also induce increased inflammation in the host through inflammasome regulation. Further, novel polymorphisms in *CARD8*, *IGRM* and *ATG16L1* are associated with GC in Chinese individuals.

O6b - Diarrheal mechanisms in Campylobacter jejuni enteritis

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Introduction: *Campylobacter jejuni* infection causes diarrhea and inflammation in humans. The objective of the present study was to characterize epithelial barrier and ion transport properties in the intestine of *C. jejuni*-infected patients. Methods: Intestinal biopsies were taken from hospitalized patients during routine endoscopy. In human colon specimens, transepithelial electrical resistance, impedance spectroscopy, electrogenic sodium transport and tracer fluxes were measured in miniaturized Ussing chambers. Results: Colonic mucosa from *C. jejuni*-infected patients showed an impairment of epithelial barrier function as indicated by a decreased transepithelial electrical resistance (32 ± 8 ohm·cm^2 versus 57 ± 6 ohm·cm^2 in healthy controls ($P<0.05$, n=5)). Paracellular permeability to fluorescein (332 Da) increased from $0.2 \pm 0.1 \cdot 10^{-6}$ cm/s in control to $2.9 \pm 0.7 \cdot 10^{-6}$ cm/s ($P<0.01$, n=5). Furthermore, a reduction of amiloride-sensitive electrogenic sodium transport from 11 ± 3 µmol/h×cm^2 in control to 1 ± 1 µmol/h×cm^2 in *Campylobacter* infection was measured ($P<0.05$). Impact of research: The pathological changes in *Campylobacter jejuni* diarrhea are characterized by epithelial barrier dysfunction and sodium malabsorption due to an impairment of the epithelial Na^+ channel (ENaC) in the colon. Thus, diarrhea in these patients is due to a leak flux as well to a malabsorptive mechanism. This study was supported by grants from the Deutsche Forschungsgemeinschaft (DFG Schu 559/11).

O7a - The human IL-17/Th17 response to Helicobacter pylori infection

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Aim: To investigate the human mucosal IL-17/Th17 (interleukin-17A/T-helper 17 cell) response to *Helicobacter pylori* (*Hp*) and its relationship with clinical and pathological outcomes. Methods: Gastric biopsies were donated by patients undergoing routine upper GI endoscopy with informed consent and ethical approval. *IFNG*, *IL17A* and *RORC2* (Th17 transcription factor) mRNA transcription were assessed by real-time PCR (RT-qPCR) relative to *GAPDH* and a pooled uninfected comparator (41 *Hp*+, 14 *Hp*-). To quantify protein expression we used Luminex cytometric bead immunoassays corrected for total protein (62 *Hp*+, 34 *Hp*-). Comparisons and correlations used non-parametric methods, reporting fold-difference in medians, and multivariate logistic regression (MLR) to adjust for age and sex. Major Findings: Infected patients had higher *IFNG* (3-fold, p=0.005, MLR p=0.302) and *IL17A* (43-fold, p<0.001, MLR p=0.018). *IL17A* was positively correlated with *IFNG* (Spearman's ρ=0.63, p=0.001) and present at 3-folder higher median concentration than *IFNG* (p=0.004). *RORC2* expression was also increased in the *Hp*+ group (3-fold, p<0.001, MLR p=0.003; 40% higher in *Hp*+ patients with peptic ulceration, p=0.046). Infected patients had increased IL-17 protein (5-fold, p<0.001, MLR p=0.004 [26pg/mg protein]; 137-fold higher than median IFNγ protein concentration, p<0.001 [<1pg/mg protein]), which was related to more severe gastric inflammation (lymphocytic: 5-fold, p=0.027; neutrophilic: 4-fold, p=0.042) but not disease. Main Conclusion and Impact: The *Hp*-infected human stomach had more *IL17A*/IL-17 than *IFNG*/IFNγ, and increased expression of the Th17 marker *RORC2*. IL-17 was associated with gastritis severity and *RORC2* was associated with peptic ulceration. This may be important for vaccine development and for predicting disease risk.

O7b - Inflammasome activation by C. jejuni

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Campylobacter jejuni efficiently invades eukaryotic cells. Intracellular bacteria are recognized by the innate immune system. Macrophages detect pathogens using pattern recognition receptors including NLRs. This subset of innate immune receptors activates the inflammasome. Inflammasome activation results in the activation of caspase-1 which confers proteolytic processing of IL-1β and IL-18 and cell death (pyroptosis). The goal of the present study was to investigate the interaction of *C. jejuni* with the inflammasome. Mouse macrophages J774.A1 cells were infected with different *C. jejuni* strains for up to 12 h. Bacterial uptake was determined by confocal microscopy and luciferase reporter assay. Real time RT-PCR showed that live *C. jejuni* and *C. jejuni* lysate caused upregulation of IL-1β mRNA levels, a precursor for inflammasome activation. Subsequent infection of these primed macrophages with *C. jejuni* resulted in the secretion of mature IL-1β into the medium, indicating inflammasome activation. Testing of several *C. jejuni* mutants for their ability to induce IL-1β secretion showed a direct correlation between the levels of invasion and inflammasome activation. Together these results indicate that *C. jejuni* activates the inflammasome in an invasion dependent fashion.

O8a - Molecular mimicry between *Helicobacter pylori* CagA and host proteins: implications for pathogenesis.

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Aims: *H. pylori* infections are associated with gastric cancer, the second leading cause of cancer death worldwide. Cytotoxin-associated antigen CagA injected by the bacteria into host cells plays a key role in pathogenesis. It is hypothesized to function as an effector protein *i.e.* to use molecular mimicry in order to hijack pleiotropic signalling pathways that regulate the cell growth and motility. Understanding of the molecular mechanisms underpinning CagA activity requires identification of the essential signalling and regulatory host proteins mimicked by CagA. Methods: We employed a combination of structural bioinformatics, limited proteolysis, molecular modeling, biophysical and X-ray crystallographic analyses to determine the boundaries of individual CagA domains, characterize their structure and identify host proteins that display sequence or structure similarity to CagA. Major Findings: Our analysis revealed significant homology of the individual CagA subdomains to the protein-protein interaction domain PDZ, pleckstrin homology (PH) domain, Shc SH2 domain, the Alix protein,

dimerization domain of human adaptor protein Skap-Hom, geminin coiled-coil domain, tropmyosin's binding site for actin, C-terminal domain of occludin and SH2-kinase linker domain of the Human tyrosine kinase ZAP-70. Conclusion and Impact of research: The results presented at the meeting will be discussed in the light of implications of the molecular mimicry between CagA and the host proteins for the mechanisms by which CagA associates with the lipid bilayer, translocates into the host cell, interacts with signalling and regulatory proteins, interferes with the assembly of functional tight junctions and induces actin cytoskeletal rearrangements.

O8b - SIGIRR-deficient mice, a novel infection model for the study of innate immune responses to *Campylobacter jejuni*

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Little is known about how *Campylobacter jejuni* triggers intestinal inflammation during the course of human infection. Answering this question has been limited due to *C. jejuni's* asymptomatic colonization of many of its hosts, including many commonly used animal models. It is believed that signaling through innate immune receptors such as TLR2 and TLR4 are one of the focal points through which *C. jejuni* can stimulate the immune system. We observed that mice deficient in SIGIRR, a negative regulator of MyD88-dependent signalling, display a hyperactive inflammatory response to a wide variety of pathogen-associated stimuli. When SIGIRR deficient mice are exposed to an oral inoculum of *C. jejuni* 81-176 following vancomycin pre-treatment, they are highly colonized with 10^9–10^{10} CFUs/g and show strong signs of acute gastroenteritis. This is in contrast to wild type C57BL/6 mice, where vancomycin pre-treatment allowed for *C. jejuni* colonization, but no signs of intestinal inflammation were evident. To define the innate receptors involved in the enteritis seen in SIGIRR deficient mice, we infected TLR2/SIGIRR and TLR4/SIGIRR double deficient mice. TLR2/SIGIRR deficient mice also suffered severe inflammation in response to *C. jejuni,* developing symptoms even more rapidly than the SIGIRR deficient mice. In contrast, TLR4/SIGIRR double deficient mice displayed only mild signs of inflammation. The reduced inflammation and pathology seen in the TLR4/SIGIRR deficient mice suggests a prominent role for TLR4 in mediating intestinal inflammation in response to *C. jejuni* infection. Additionally, these results suggest that SIGIRR-deficient mice will provide a valuable model of *C. jejuni* infection and pathogenesis.

O9a - Effects of *Helicobacter pylori* on host immunity and gut microbiome using the C57/Bl6 mouse model.

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Although *Helicobacter pylori* (*Hp*) is the major cause of gastric cancer and peptic ulcer disease in middle aged and elderly humans, there is increasing evidence that *Hp* might protect against diarrheal disease, childhood-onset asthma and reflux esophagitis. Thus, despite its late-in-life pathogenicity, with potential early-life benefits, *Hp* might be considered a commensal. Its presence in the human microbiome is currently disappearing and little is known how *Hp* influences the host's microbiome and immunity. In this study, we challenged 4-week-old (4W) and 6-week-old (6W) C57/Bl6 mice with *cagA*+ *Hp* PMSS1. Groups of mice were serially sacrificed over a period of 6-months with multiple pre-mortem blood and fecal samples. Challenged mice were *Hp* positive as verified upon sacrifice by PCR and culture ($\approx 10^3$–10^6 cfu/g stomach). *Hp* was not detected in fecal pellets. Isolated strains from each mouse were stored for further analysis including whole genome sequencing. Blood from 6W-mice was assayed for IgM and IgG to *Hp* and CagA. All mice displayed IgM/IgG to *Hp* but not to CagA. Stomach, lung, spleen, and intestinal samples were frozen in RNA-protecting reagents for further analysis. Extracted DNA from fecal pellets, ileal and cecal content were subjected to 16S rRNA gene pyro-sequencing to determine changes in the gut microbiome due to *Hp* colonization. Flow-cytometry was performed on spleens and lungs. In the lung, significantly higher levels of Th17-cells were observed in 4W-mice compared to the control group. Further analysis of the collected specimens will allow us to investigate the interactions of *Hp* with its host.

O9b - A role for Siglecs in the recognition of Guillain-Barré syndrome-related *Campylobacter jejuni* strains

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Introduction: Sialylated epitopes on *Campylobacter jejuni* lipooligosaccharides (LOS) can induce an antibody response that leads to the development of the immune-mediated neuropathies Guillain-Barré syndrome (GBS) and Miller Fisher syndrome (MFS). GBS is characterized by general limb paresis and the presence of auto-antibodies with specificity for gangliosides GM1 and GD1a. MFS is characterized by oculomotor weakness and antibodies directed to ganglioside GQ1b. Epitope recognition is an important first step in the induction of an antibody response. To gain insight into the immune events that lead to GBS and MFS development, we aimed to identify whether members of the sialic acid binding immunoglobulin-like lectin (Siglec) family are able to recognize sialylated GBS- and MFS-associated *C. jejuni* strains. Methods: Siglec binding and functional consequences of binding were assessed using ELISA, Siglec-expressing CHO- and THP-1 cells and primary human macrophages. Results. We demonstrate that monosialylated, GBS-associated *C. jejuni* strains preferentially bind to sialoadhesin (Sn, Siglec-1) whereas disialylated, oculomotor weakness- and MFS-associated strains bind to Siglec-7. Using purified *C. jejuni* LOS, nonsialylated *C. jejuni* strains and sialic acid knockout mutants, we show that *C. jejuni* binding to either Sn or Siglec-7 is LOS and sialic acid specific. Sn binding enhances *C. jejuni* uptake and increases the production of interleukin-6 by primary human monocyte-derived macrophages. Siglec-7 binding correlates with the presence of anti-GQ1b antibodies in patient serum. Impact. LOS-specific binding of *C. jejuni* to Sn or Siglec-7 may be initiating events in immune recognition and contribute to anti-ganglioside antibody production and the development of GBS and MFS.

O10a - Gastric colonization with a restricted commensal flora replicates the promotion of neoplastic lesions by diverse intestinal flora in the *Helicobacter pylori* INS-GAS mouse model of gastric carcinogenesis

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Aims: Gastric colonization with intestinal flora (IF) may promote *Helicobacter pylori* (*Hp*) associated gastric cancer. It is unknown if the mechanism involves colonization with specific or diverse microbiota secondary to gastric atrophy. Methods: Gastric colonization with Altered Schaedler's flora (ASF) and *Hp* were correlated with pathology, immune responses and mRNA expression for inflammatory and cancer-related genes in germfree (GF), monoassociated (m*Hp*), restricted ASF (rASF; 3 species), and specific pathogen-free (complex IF), hypergastrinemic INS-GAS mice. Findings: INS-GAS mice colonized with rASF, IF or *Hp* alone had mild to moderate gastric lesions 7 months post infection. Male mice co-colonized with rASF*Hp* or IF*Hp* developed the most severe pathology. IF*Hp* males had the highest inflammatory responses and 40% developed invasive gastrointestinal intraepithelial neoplasia (GIN). Notably, rASF*Hp* colonization was sufficient to cause GIN in 23% of male mice along with inflammatory biomarkers. Enhanced gastritis in male rASF*Hp* mice was also accompanied by decreased gastric bacterial colonization and an overgrowth of *Lactobacillus murinus* (ASF361), suggesting commensals promote gastritis. *Hp* colonization independently elevated expression of *IL-11*, the prostaglandin and cancer-related gene *Ptger4*, and *Tgf-b*, further supporting a role for *Hp* infection in the acceleration of gastric cancer in INS-GAS mice. Conclusions: rASF was comparable to IF in promoting the progression of *Hp* gastritis to GIN in male INS-GAS mice, indicating gastric colonization efficiency is more important than IF diversity. Impact: rASF or IF colonization with *Hp* reproduced key features of gastric adenocarcinoma observed in patients with advanced gastric atrophy secondary to *Hp* gastritis.

O10b - *Campylobacter jejuni* at the host pathogen interface: The role of periplasmic chaperones in the biogenesis of outer membrane proteins

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Campylobacters are the leading cause of human bacterial gastroenteritis around the world. The outer membrane (OM) is the most important factor in their interaction with the host; it acts in adhesion, interaction with the immune system, cell signalling and as a nutrient barrier. The mechanism by which OM proteins are transported and correctly assembled in the OM is not fully understood, but depends upon periplasmic chaperones, which have not been well studied in campylobacters. Here, we investigated the role of periplasmic chaperones in C. jejuni NCTC 11168, focusing on Cj0694, a PpiD homologue which may have a chaperone role for periplasmic proteins or the translocation of OMPs across the periplasm. We examined the biochemical functions of this protein, and the phenotypic characterisation of a null mutant in the cognate gene. Cj0694 was successfully overexpressed in E. coli as a his-tagged protein and purified by Ni-NTA and ion-exchange chromatography. Chaperone domain activity was clearly detected in aggregation assays using the model protein rhodanese, and its peptidyl-prolyl cis/trans isomerise (PPIase) domain activity was demonstrated by an enhanced refolding rate of denatured ribonuclease T1 in fluorescence recovery assays. Evidence that Cj0694 plays a role as a periplasmic chaperone in C. jejuni was suggested by an altered accumulation of proteins in the periplasm of the cj0694 null mutant. Proteomic analysis of OM and periplasmic preparations using 2D-gels and mass spectrometry were used to identify client proteins.

O11a - The Potential of Dextran-Based Glycoconjugates for Development of *Helicobacter pylori* Vaccine

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Aims: We have recently demonstrated that synthetic glycoconjugates based on a truncated lipopolysaccharide (LPS) of *Helicobacter pylori* and containing an α-1,6-glucan chain induced broadly cross-reactive functional antibodies in immunized animals. To investigate the candidacy of α-1,6-glucan as an alternative vaccine strategy we prepared glycoconjugates based on dextrans produced by lactic acid bacteria *Leuconostoc mesenteroides* B512F and consisting of linear α-1,6-glucan chains with limited branching. Methods: Three dextrans with molecular masses of 5,000 Da, 3,500 Da and 1,500 Da, respectively, were modified with a diamino group-containing linker and conjugated to a carrier protein, tetanus toxoid (TT), and their immunological properties investigated. Major Findings: The conjugates were immunogenic in both mice and rabbits and induced specific IgG responses against α-1,6-glucan-expressing *H. pylori* LPS. Studies performed with post-immune sera of mice and rabbits immunized with dextran-based conjugates demonstrated cross-reactivity with LPS from typable and non-typable *H. pylori* strains and selected mutants. The post-immune sera from rabbits that received the conjugates exhibited functional activity against α-1,6-glucan-positive strains of *H. pylori*. Main Conclusion: These studies demonstrate that dextran-based synthetic glycoconjugates presenting a linear α-1,6-glucan epitope induce functional immune responses to *H. pylori*. Impact of research: Dextran-based glycoconjugates may offer a simplified approach to the development of a carbohydrate-based vaccine against *H. pylori*.

O11b - *Campylobacter jejuni* induces acute non-self-limiting enterocolitis in gnotobiotic IL-10-/- mice via Toll-like-receptor-2 and -4 signalling

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Aim: To present a murine *C. jejuni* infection model displaying acute non-self-limiting enterocolitis mimicking severe episodes of human campylobacteriosis and to elucidate potential Toll like receptor (TLR) -2 and/or –4 dependence of immunopathology. Methods and Major Findings: Gnotobiotic IL-10-/- mice were generated by quintuple antibiotic treatment starting right after weaning, thereby preventing animals from commensal bacteria induced colitis. Following oral infection, *C. jejuni* B2 strain readily colonized the gastrointestinal tract of gnotobiotic IL-10-/- mice and induced acute non-self-limiting ulcerative enterocolitis within 7 days. Immunopathology was further characterized by increased numbers of apoptotic cells, T- and

B-lymphocytes as well as regulatory T-cells and elevated TNF-a, IFN-g, and MCP-1 concentrations in the inflamed colon. Infection of gnotobiotic IL-10-/- mice with a commensal *E. coli* strain, however, did not induce disease indicating a *C. jejuni*-specific induction of disease. *C. jejuni* infection of gnotobiotic IL-10-/- mice additionally lacking TLR-4 or -2 revealed that immunopathology is mediated by TLR-4- and, less distinctly, by TLR-2-dependent signalling of *C. jejuni*-LPS and -lipoprotein, respectively. Main Conclusion and Impact of the research: The presented murine *C. jejuni* infection model displaying acute non-self-limiting enterocolitis mimics severe episodes of human campylobacteriosis as in immuno-compromized patients. This acute model proves useful for further dissecting the immunopathological mechanisms underlying *Campylobacter* infections *in vivo* and to elucidate the interplay between intestinal pathogens, the commensal intestinal microbiota and the innate as well as adaptive immune system of the host.

O12a - Use of a novel ex *vivo* three-dimensional system to define host-microbial interactions with carcinogenic potential.

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Gastric adenocarcinoma is the second leading cause of cancer-related death in the world and *Helicobacter pylori* is the strongest known risk factor for this malignancy. Contact between *H. pylori* and gastric epithelial cells is critical for inducing injury; however, most *in vitro* models fail to recapitulate events occurring within the gastric niche. Murine gastric organoids are three-dimensional, single-layered epithelial organ-like structures derived from stem cells, and provide unique opportunities to study host-*H. pylori* interactions in a pre-clinical model. Using immunofluorescence with antibodies directed against gastric mucin, H^+K^+ATPase, gastrin, TFF2, somatostatin and chromogranin A, we found that organoids derived from whole stomach develop into a self-organizing differentiation axis containing mucus cells, parietal cells, G-cells, mucus neck cells, D-cells and ECL cells respectively. Addition of EGF to gastric organoids resulted in phosphorylation of EGFR indicating organoids are responsive to ligands implicated in gastric carcinogenesis. To define the influence of microbial elements, we infected organoids with the *cag*$^+$ carcinogenic *H. pylori* strain 7.13 via luminal microinjection and, by confocal immunofluorescence microscopy, demonstrated mislocalization of the tight junction protein occludin, and increased proliferation as assessed by EdU staining. CagA augments gastric cancer risk; therefore, we microinjected gastric organoids with strain 7.13 or its isogenic *cagA*$^-$ mutant. Proliferation was significantly higher in organoids infected with wild-type 7.13 compared to the *cagA*$^-$ mutant. These results indicate that gastric organoids are a novel model that can provide important insights into molecular interactions with carcinogenic potential that occur between *H. pylori* and epithelial cells within the gastric niche.

O12b - Meaningful Dissemination of Community-Driven H. pylori Microbiology Research in Indigenous Arctic Communities

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The Aklavik *H. pylori* Project, a community-driven project led by the Canadian *Helicobacter pylori* (CAN*Help*) Working Group, brings together health care providers, community members and researchers to address community concerns regarding the association of *H. pylori* with stomach cancer. The aim of this study was to have community members participate in the design of data dissemination materials and for researchers to learn more about daily life and culture in Aklavik. Two researchers (SC, MK) travelled to Aklavik to meet with community members and organizations to share microbiology results, recruit youth for the exchange project and experience life in Aklavik. The two recruited youth community members (BLK, PDE) travelled to Edmonton, AB to visit the CAN*Help* microbiology lab to learn about and observe the microbiology methods used to study the antimicrobial susceptibility and genetic characteristics of the *H. pylori* bacteria, and also how to interpret the data. They also met with and observed the work of public health researchers in the CAN*Help* offices. Upon return to Aklavik, they guided grade 10 science students in developing dissemination materials for educating other community members about *H pylori* microbiology research. The classroom activities augmented and reinforced concepts from the general science curriculum, and explored how local cultural values and knowledge can be incorporated into the community-based scientific research. The dissemination materials developed in this knowledge exchange program allows for sustained

contact between community members and researchers, and will be used to help present the Aklavik *H. pylori* Project results to other interested Arctic communities.

O13a - *Campylobacter jejuni* infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses

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Aim: To overcome colonization resistance in infant mice and characterize potential extra-intestinal sequelae in asymptomatic long-term carriers following *C. jejuni* infection. Methods and Major Findings: Whereas adult mice (3 months of age) harbouring a conventional intestinal microbiota display strong colonization resistance against *C. jejuni*, we here show that upon infection right after weaning, 3-weeks-young infant mice were readily colonized by *C. jejuni* B2 strain for more than 100 days. Within six days following infection, mice developed acute enterocolitis as indicated by bloody diarrhoea, colonic shortening, and increased apoptotic cell numbers in the colonic mucosa. Similar to human campylobacteriosis clinical manifestations were self-limited and disappeared within two weeks. Interestingly, long-term *C. jejuni* infection was accompanied by distinct intestinal immune and inflammatory responses displayed by asymptomatic mice as indicated by increased numbers of T- and B-lymphocytes, regulatory T-cells, neutrophils, as well as apoptotic cells in the colonic mucosa. Strikingly, *C. jejuni* infection also induced a pronounced influx of immune cells into extra-intestinal sites such as liver, lung, and kidney. Main Conclusion and Impact of the research: These results support the impact of the age-dependent microflora composition in CR against *C. jejuni* and demonstrate that the infant mouse model resembles *C. jejuni* mediated immunopathogenesis including self-limited enterocolitis in human campylobacteriosis. Furthermore, potential clinical and immunological sequelae of chronic *C. jejuni* carriers in humans can be further elucidated by investigation of long-term infected infant mice. The observed extra-intestinal disease manifestations might help to unravel the mechanisms causing complications such as reactive arthritis or Guillain-Barré-Syndrome.

O13b - Sticky and Sweet: The Interaction of *Campylobacter jejuni* and Helicobacter pylori with purified mucins

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Background: Mucus plays a crucial role in bacterial colonization of host cell surfaces. *Helicobacter pylori* and *Campylobacter jejuni* colonise stomach and intestinal mucus, respectively. *H. pylori* binding to gastric mucin has been extensively studied but little information exists on the carbohydrate/lectin pairs involved in mediating *C. jejuni* infection. While individual oligosaccharides are used to study the microbes interacting with glycan receptors they do not reflect the density and complexity of mucin glycans. We investigated binding of *H. pylori* and *C. jejuni* to a panel of native mucins from animals and from colonic cell lines using a novel mucin microarray platform that enables generation of quantitative binding data. Results: Both organisms bound to a distinctly different subset of mucins. *C. jejuni* showed a clear tropism for chicken mucin, with the strength of the interaction dependent on mucin origin (large intestine>small intestine>caecum). Binding was consistent across different isolates and comparable at 37°C and 42°C. *H. pylori* displayed appreciable binding to porcine stomach mucin, although the intensity of the interaction was less than that of *C. jejuni* to chicken mucin. Binding of both organisms to mucins from cell lines correlated with the complexity of the mucin glycans. Conclusion: The strong interaction between *C. jejuni* and chicken mucin may prevent bacteria interacting with epithelial cells thus explaining the commensal nature of the organism in chickens. Results suggest mucin glycans likely mediate the tropism that these bacteria exhibit for different ecological niches. Natural mucins are powerful probes to identify novel bacterial lectins and host receptors.

O14a - Intestinal microbiota composition of interleukin-10 deficient C57BL/6J mice and susceptibility to *Helicobacter hepaticus*-induced colitis

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The mouse pathobiont *Helicobacter hepaticus* can induce typhlocolitis in interleukin-10 deficient mice, and *H. hepaticus* infection of immunodeficient mice is widely used as a model to study the role of pathogens and commensal bacteria in the pathogenesis of inflammatory bowel disease. C57BL/6J *Il10*$^{-/-}$ mice kept under specific pathogen-free conditions in two different facilities (MHH and MIT), displayed strong differences with respect to their susceptibilities to *H. hepaticus*-induced intestinal pathology. Mice at MIT developed robust typhlocolitis after infection with *H. hepaticus*, while mice at MHH developed no significant pathology. We hypothesized that the intestinal microbiota might be responsible for these differences and therefore performed high resolution analysis of the intestinal microbiota composition in uninfected mice from the two facilities by deep sequencing of partial 16S rRNA amplicons. The microbiota composition differed markedly between mice from both facilities. Significant differences were also detected between the two groups of MHH mice. Of the 119 non-rare operative taxonomic units (OTUs) identified in the dataset, 24 were only found in MIT mice, and another 13 OTUs could only be found in MHH samples. While most of the MHH-specific OTUs could only be identified to class or family level, the MIT-specific set contained OTUs identified to genus or species level, including the opportunistic pathogen *Bilophila wadsworthia*. The susceptibility to *H. hepaticus*-induced colitis differed considerably between *Il10*$^{-/-}$ mice originating from the two institutions. This was associated with significant differences in microbiota composition, highlighting the importance of characterizing the intestinal microbiome when studying murine models of IBD.

O14b - CagA-dependent down regulation of microRNA-320 by carcinogenic *Helicobacter pylori* promotes expression of the cell survival protein, Mcl-1 *in vitro and in vivo*

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Helicobacter pylori strain 7.13 induces gastric cancer in rodents and was derived from a human clinical isolate, B128. The parental strain B128 induces inflammation in animals, but not cancer, thus providing a unique opportunity to define mechanisms that mediate gastric carcinogenesis. Host microRNAs (miRNAs) can function as either oncogenes or tumor suppressors and are frequently dysregulated in cancer. To identify miRNAs involved in *H. pylori*-mediated gastric carcinogenesis, miRNA microarray analyses were performed with RNA from gastric epithelial cells co-cultured with strain B128 or 7.13. Among the 61 differentially expressed miRNAs, the tumor suppressor miR-320 was down regulated by strain 7.13. miR-320 negatively regulates Mcl-1, a member of the Bcl-2 family that functions to inhibit apoptosis and, consistent with down regulation of miR-320, strain 7.13 significantly induced Mcl-1 expression. To define the effect of the *H. pylori* oncoprotein CagA, gastric epithelial cells were co-cultured with a 7.13 *cagA*$^{-}$ isogenic mutant. Loss of *cagA* significantly attenuated both the reduction of miR-320 and the increase in Mcl-1 induced by wild-type strain 7.13. To extend these results, mice were challenged with strain 7.13 or the *cagA*$^{-}$ mutant and, consistent with the *in vitro* data, *H. pylori* induced Mcl-1 expression in a *cagA*-dependent manner. Findings from human biopsies confirmed these results by demonstrating that *H. pylori* up regulates Mcl-1 expression in a *cagA*-dependent manner and that levels of Mcl-1 expression parallel the severity of gastric pre-malignant lesions. These findings suggest that down regulation of miR-320 and subsequent up regulation of Mcl-1 by *H. pylori* likely plays a role in carcinogenesis.

O15a - A comprehensive overview of *Campylobacter* and Helicobacter in de-novo Paediatric Inflammatory Bowel Disease.

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Background: Children presenting for the first time with inflammatory bowel disease (IBD) offer a unique opportunity to study aetiological agents before the confounders of treatment. Microaerophilic bacteria can exploit the ecological niche of the intestinal epithelium; *Helicobacter* and *Campylobacter* are previously implicated in IBD pathogenesis. Aim: To study these microaerophilic bacteria in de-novo paediatric IBD. Subjects and Methods: 100 children undergoing colonoscopy were recruited including 44 treatment naïve de-novo IBD patients and 42 with normal colons. Colonic biopsies were subjected to microaerophilic culture with Gram-negative isolates then identified by sequencing. Biopsies were also PCR screened for the specific microaerophilic bacterial groups: Helicobacteraceae and Campylobacteraceae. Major Findings: 129 Gram-negative microaerophilic bacterial isolates were identified from 10 genera. Unusual *Campylobacter* were isolated from 8 subjects (including 3 *C. concisus*, 1 *C. curvus*, 1 *C. lari*, 1 *C. rectus*, 3 *C. showae*). No *Helicobacter* were cultured. When comparing IBD vs. normal colon control by PCR the prevalence figures were not significantly different (*Helicobacter* 11% vs. 12%, p=1.00; *Campylobacter* 75% vs. 76%, p=1.00. Main Conclusions and Impact of Research: This study offers a comprehensive overview of the microaerophilic microbiota of the paediatric colon including at IBD onset. *Campylobacter* appear to be surprisingly common, are not more strongly associated with IBD and can be isolated from around 8% of paediatric colonic biopsies. *Helicobacter* species are relatively rare in the paediatric colon.

O15b - The cytolethal distending toxin of Helicobacter pullorum targets vinculin and cortactin, and triggers formation of lamellipodia in intestinal epithelial cells

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Helicobacter pullorum, a bacterium initially isolated from poultry, is an emerging pathogen in human digestive diseases. However, very few studies have been carried out on the virulence of *H. pullorum*. The effects of the cytolethal distending toxin (CDT) of *H. pullorum* were investigated by a two- complementary original system based on the development of 1) a *CdtB* isogenic *H. pullorum* strain and 2) a lentiviral expression system to express the active CdtB subunit directly into the epithelial cells (Caco-2, HCA7 and HT-29). In addition to the well-known effects of the CDT (cellular distending phenotype, actin cytoskeleton remodelling and G2/M cell arrest), the CdtB induced an atypical delocalization of vinculin from focal adhesions to the perinuclear region, the formation of cortical actin-rich large lamellipodia with an upregulation of cortactin, and decreased cellular adherence. In conclusion, the CDT of *H. pullorum* is responsible for major cytopathogenic effects *in vitro*, confirming its role as a main virulence factor of this emerging human pathogen.

O16a - Particular lipooligosaccharide loci and capsule types co-occur in Guillain-Barré syndrome-associated Campylobacter jejuni strains

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Introduction: The frequent detection of *C. jejuni* strains with sialylated lipooligosaccharides (LOS) in stools of patients with uncomplicated enteritis but low incidence of post-infectious Guillain-Barré syndrome (GBS) and Miller Fisher syndrome (MFS) implies that additional bacterial factors are involved in the development of these diseases. Methods: The prevalence

of eight LOS gene loci and nine genes encoding structures known to be involved in *C. jejuni* virulence was determined on a collection of 37 strains isolated from stool samples of patients with GBS/MFS and 143 strains isolated from stool samples of patients with uncomplicated enteritis. Additionally, a multiplex PCR recognizing 17 major capsule loci was performed. Results: LOS genotyping confirmed and strengthened the earlier described associations between LOS class A and GBS, and LOS class B and MFS. The LOS class A1 and B2 alleles were the most common alleles among LOS class A and B strains (each ~ 80%) from GBS, MFS and uncomplicated enteritis patients. Capsule genotyping revealed a GBS/MFS-related correlation between LOS class and capsule type. The main LOS class/capsule type combinations that were observed in this collection were LOS class A combined with capsule type HS1, HS2, HS4 or HS19, LOS class B combined with capsule type HS2 or HS4, and LOS class C combined with capsule type HS1 or HS2. Impact: We conclude that, in combination with LOS locus classes that contain genes involved in LOS sialylation, capsule types HS1, HS2, HS4 and HS19 are frequently associated with the development of GBS and MFS.

O16b - *H. pylori* CagN, a protein which targets human ubiquitin and related small modifiers

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Helicobacter pylori is able to chronically persist within its host and therefore requires efficient strategies to guide the immune responses and its own survival. Immunoevasive and immunomodulatory mechanisms of several pathogenic bacteria were shown to target the host ubiquitin-proteasome system (UPS), which regulates protein degradation, cell signaling, immune response and other essential cellular processes. So far, little is known about the potential of *H. pylori* to interfere with the host UPS. The *H. pylori cag* pathogenicity island (*cag*PAI) is associated with disease severity and carcinogenesis, suggesting that *cag* functions interfere with immune responses and cellular signaling. Using BLAST searches, unbiased specific pattern searches, and hidden Markov models to identify short catalytic residues from different DUB families in a specific DUB database, we obtained several interesting hits among *H. pylori cag*PAI proteins which possibly interact with the host UPS. In functional assays, we identified *H. pylori* CagN as one candidate protein of the *cag*PAI, which possesses deubiquitinylating and deneddylating activity. CagN targeted the *H. pylori* oncoprotein CagA for proteolytic degradation, and led to an overall decrease of a subset of cellular ubiquitin. These results are a basis for further investigations of host cell modulation by *cag*PAI-positive *H. pylori* strains.

O17a - A randomized, double-blind, placebo-controlled trial of azithromycin in *Campylobacter concisus* positive patients with diarrhoea

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Introduction: *Campylobacter concisus* has recently been reported to have a high incidence in the Danish population almost equal to *Campylobacter jejuni*. The main clinical symptom is prolonged watery diarrhoea. Whether patients with *C. concisus* gastroenteritis may benefit from antibiotic treatment is unknown. This is the first clinical trial with *C. concisus* infected diarrheic patients. Methods: A randomized, double-blind, placebo-controlled clinical trial(phase 3) of azithromycin (500 mg once daily for 3days) in *C. concisus* positive adult patients with diarrhoea was started in March 2012 and still recruit until December 2013. For the full in- and exclusion-criteria, please see ClinicalTrials.gov; Identifier: NCT01531218, but the main reasons for exclusion are: If patients has received antibiotics; has a co-pathogen in their faecal sample; has a chronic gastrointestinal disease; or has diarrhoea more than three weeks. The primary end-points are number of stools per day during a ten day follow-up period, as well as the development of the following major symptoms: abdominal pain, nausea, vomiting and fever. Results and impact of research: During the first 12 months 5,658 stool samples from 4,066 patients were cultured for *C. concisus* as well as other pathogenic enteric bacteria. 277 patients with *C. concisus* were identified and screened for participation. Due to the strict inclusion- and exclusion-criteria only 20 patients have been enrolled in the study, but hopefully more patients will be recruited. This is the first clinical trials in *C. concisus* positive patients, and will further clarify whether *C. concisus* infected patients should be treated with azithromycin.

O17b - Intestinal microbiota shifts towards elevated commensal Escherichia coli loads abrogate colonization resistance against *Campylobacter jejuni* in mice

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Aim: To dissect factors abrogating colonization resistance against *C. jejuni* in murine intestinal inflammation and infancy. Methods and Major Findings: In contrast to healthy adult animals *C. jejuni* stably colonized mice suffering from acute small or large intestinal inflammation. Strikingly, in mice with *Toxoplasma gondii*-induced ileitis, *C. jejuni* disseminated to mesenteric lymph nodes, spleen, liver, kidney, and blood. Furthermore, in 3-weeks-old infant mice harboring a conventional microbiota *C. jejuni* infection induced self-limiting ulcerative enterocolitis. Mice suffering from intestinal inflammation and *C. jejuni* susceptible infant mice displayed characteristic microbiota shifts dominated by increased numbers of commensal *Escherichia coli*. To further dissect the pivotal role of those distinct microbiota shifts in abrogating colonization resistance, we investigated *C. jejuni* infection in healthy adult mice in which the microbiota was artificially modified by feeding live commensal *E. coli*. Strikingly, in animals harboring supra-physiological intestinal *E. coli* loads, colonization resistance was diminished and *C. jejuni* infection induced enterocolitis mimicking key features of human campylobacteriosis. Main Conclusion and Impact of the research: Murine colonization resistance against *C. jejuni* is abrogated by changes in the microbiota composition towards elevated *E. coli* loads during intestinal inflammation as well as in infant mice. Intestinal inflammation and microbiota shifts thus represent potential risk factors for *C. jejuni* infection. Corresponding interplays between *C. jejuni* and microbiota might occur in human campylobacteriosis. Murine models introduced here mimic key features of human campylobacteriosis and allow for further analysis of immunological and molecular mechanisms of *C. jejuni* – host interactions.

O18a - Critical role of a putative lytic transglycosylase in β-lactam resistance in *Campylobacter jejuni*

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β-lactam antibiotics are an important class of antibiotics for treating bacterial infections. Emergence of β-lactam resistance has greatly compromised clinical effectiveness of this group of antibiotics. Despite prevalent β-lactam resistance in *Campylobacter jejuni*, the leading bacterial cause of human diarrhea in developed countries, molecular basis of β-lactam resistance in *C. jejuni* is still largely unknown. In this study, *C. jejuni* 81–176 was used for random transposon mutagenesis. Screening of a 2,800-mutant library identified 22 mutants with increased susceptibility to ampicillin. Of these mutants, 20 mutants were inserted in CmeABC multidrug efflux pump while the other 2 contains mutations in *Cj0843c* (a putative lytic transglycosylase gene) and its upstream gene *Cj0844c*, respectively. Complementation experiment demonstrated that Cj0843 contributes to β-lactam resistance. The *Cj0843c* insertional mutation was subsequently introduced to diverse *C. jejuni* clinical strains; MIC test showed that Cj0843c contributes to both intrinsic and acquired β-lactam resistance of *C. jejuni*. In addition, inactivation of Cj0843c dramatically reduced β-lactamase activity, indicating that Cj0843c could modulate the production of β-lactamase. High purity recombinant Cj0843 was produced for generation of specific antiserum. The Cj0843 was localized in periplasm, as demonstrated by immunoblotting using specific antibodies. Genomic examination and PCR analysis showed *Cj0843c* is prevalent and conserved in *C. jejuni*, *C. coli*, and *Helicobacter pylori*. Together, this study identifies a new mechanism of β-lactam resistance in *C. jejuni* and provides insights into the molecular basis of β-lactamase induction by the liberated murein fragments.

O18b - PgdA deacetylase regulates evasion of host defense mechanisms by *Helicobacter pylori* that contribute to bacterial persistence

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Helicobacter pylori (Hp) is predominantly an extracellular pathogen that is typically juxtaposed to gastric epithelial cells (GEC); however intracellular bacteria have been identified *in vitro* and *in vivo*. Acetylation and deacetylation of microbial

constituents is associated with biofilm formation and host immune evasion. The *Hp pgdA* gene encodes a peptidoglycan deacetylase; therefore, we defined novel phenotypes mediated by *pgdA* within the carcinogenic *Hp* strain 7.13. Using confocal microscopy and fluorimetry, we demonstrated that WT *Hp* had a significantly higher density of outer membrane-associated polysaccharide than the *ΔpgdA* strain ($p<0.05$). In contrast to planktonic morphology exhibited by the WT strain, the *ΔpgdA* mutant formed compact bacterial aggregates and exhibited motility defects within soft-agar ($p<0.001$). Transmission electron microscopy revealed that the WT and *ΔpgdA* mutant strains both formed zipper-like structures during co-culture with GEC; however, the *ΔpgdA* mutant was phagocytosed more efficiently, leading to a higher density of mutant bacteria within endocytic vacuoles. To determine whether intracellular presence of *Hp* corresponded to viability, we performed gentamycin protection assays and observed that the numbers of viable *ΔpgdA* mutant bacteria recovered were significantly reduced when compared with the WT strain ($p<0.001$). To extend these results *in vivo*, we infected Mongolian gerbils with the WT and *ΔpgdA* strains. Colonization efficiency and density were substantially decreased in gerbils challenged with the *ΔpgdA* mutant versus the WT strain, 2 weeks post-inoculation. Collectively, these results indicate that PgdA deacetylase plays an important role in bacterial fitness, potentially enhancing the resistance to phagocytosis, intracellular degradation and immune clearance

O19a - Impact of Rearing Conditions on Arsenic Resistance in *Campylobacter spp.*

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Roxarsone, a coccidiostat used in poultry production, is processed by litter microflora to the harmful inorganic arsenic form. This may be re-ingested by the bird causing the gut microflora to become exposed and subsequently select for resistance to inorganic arsenic. Given that most conventional systems use roxarsone, while organic systems do not, we hypothesized that *Campylobacter* isolated from conventional birds will be more resistant to arsenic compounds than isolates from organic birds. Eighty-two *Campylobacter* isolates from retail chickens, organic and conventional, were screened for arsenic resistance. PCR primer sets specific for the arsenic resistance genes *arsC* and *acr3* were used to determine the presence/absence of these genes in the isolates. Minimum inhibitory concentrations (MICs) for arsenite, arsenate, and roxarsone were determined for each isolate using the NCCLS agar dilution susceptibility testing method. It was determined that, among the 38 conventionally raised isolates, 55% possessed both arsenic resistance genes, while among the 44 organically raised isolates, only 27% possessed both arsenic genes. The difference in possession of these genes was significant ($p<0.05$). The median arsenite, arsenate, and roxarsone MICs for the organic isolates positive for the arsenic genes were 4ug/mL, 128ug/mL, and 32ug/mL. While, the median arsenite, arsenate, and roxarsone MICs for conventional positive isolates were 16ug/mL, 256ug/mL, and 64ug/mL, respectively. From our results, it is clear that within the subset of *Campylobacter* isolates containing arsenic resistance genes, those from conventionally reared birds possessed greater resistance to arsenic compounds than those from organically reared birds.

O19b - Glycosylated moieties' of *Campylobacter jejuni* flagella modulate Dendritic Cell IL-10 expression via Siglec-10 receptor engagement

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Aims: *Campylobacter jejuni,* a commensal in poultry members, causes acute gastroenteritis in humans. Understanding of *Campylobacter jejuni* pathogenesis lags far behind that of other gastrointestinal pathogens despite *Campylobacter sp.* being a leading cause of bacterial gastroenteritis in the developed world. Here, we wish to determine how *C. jejuni* modulates host innate IL-10 axis; an axis that may promote carriage and/or pathology. Methods: Murine and Human dendritic cells (DCs) were co-cultured with *C. jejuni* strains and infection-mediated DC signalling events that regulate IL-10 expression were identified using a combination of gene knock-out cells and inhibitors. Siglec-10 Receptor expressing CHO cells allowed delineation of its role in *C. jejuni*-driven DC IL10. Major Findings: It is well established that *C.jejuni* flagella has lost its ability to recognise TLR5. In the present study we found the glycan moeities' of *C. jejuni* flagella to interact with a member of the Siglec family of receptors, the Siglec-10 receptor. This interaction led to potent modulation of IL-10 expression which was completely reliant on MyD88 engagement and p38 signalling. Conclusion: We propose that the glycan structures of *C. jejuni* flagella interact with host innate cells and specifically target host IL-10 axis. These host-pathogen interactions present a novel immune evasion strategy; a strategy that may aid in successful colonisation and subsequent disease manifestation.

O20 - Helicobacter species of concern: Crystal ball predictions for the next decade.

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Over the last three decades, evidence has accrued to support the role of a range of *Helicobacter* species in the aetiology of gastrointestinal disease in humans and animals. While the aetiological role of *Helicobacter pylori* in gastritis, peptic ulcer disease and gastric cancer is well established, this bacterium is likely to remain a major species of concern over the coming decade. Given the multifactorial nature of gastric cancer and geographic differences in both *H. pylori* strains and host genetic markers, the identification of biomarkers that can determine those at risk of gastric cancer has proven difficult, as has the development of an *H. pylori* vaccine. Given this, studies investigating these issues are likely to continue to be a focus of on going research. Further, increasing reports of potential associations between *H. pylori* and a range of non-gastric human diseases, some of which are now backed by fairly strong epidemiological evidence, will make this a further important area of research, particularly in relation to determination of pathogenic mechanisms involved. Furthermore, recent data showing links between the composition of the gastric microbiota and variable disease outcomes of *H. pylori* infection in mice, as well as reports supporting a link between *H. pylori* and colorectal cancer are likely to stimulate significant research interest in these areas. Increasing reports of the role of a range of enterohepatic *Helicobacter species* in the aetiology of human disease, including gastroenteritis, hepatobiliary disease and IBD, as well as the involvement of both gastric and enterophepatic *Helicobacter* species in animal disease, will also make these an important focus of research over the next decade.

O21 - Plenary – *Campylobacter*

Rob Mandrell

O22a - What have we learnt of the genus Arcobacter since its description in 1991?

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The taxonomy of the genus *Arcobacter*, defined in 1991 with 2 species has evolved to 17 species, and many more are pending to be described based on the analyses of bacterial communities with the 16S rRNA gene. It has been discovered that shellfish, is an important reservoir for new species, five have been described from this habitat, however, other new species came from animals. *Arcobacter* spp. have been isolated from different types of food like meat products, milk etc. as well as from water. In 3 waterborne outbreaks, bacteria of this genus were recovered from the drinking water and/or from the faeces of the patients with diarrhoea. A clear correlation between the concentration of bacteria indicators of faecal pollution in water and the presence of Arcobacter has been demonstrated. These findings have been corroborated with the persistence of these bacteria in waste water treatment plants in different studies. The complete genomes of some species have revealed the presence of several putative virulence genes for which primers have been designed and used to study their presence in several species. Alterations of the blood cell counts in experimental infections have been demonstrated, being this finding additional pathogenicity evidence. New clinical cases of bacteremia and other extraintestinal and intestinal infections have been described. However, the main limitation still is the lack of routine systematic analysis for these bacteria in humans and animals. Further gaps on the knowledge on this still poorly known group of bacteria will be presented.

O22b - Rapid, alignment-free analysis of whole genome sequences of *Campylobacter jejuni* and *Campylobacter coli* for molecular epidemiology

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Introduction: Current methods for molecular typing of clinically important *Campylobacter* (MLST, PFGE, CGF) only use a small part of the genome and lack resolution and discriminatory power. Developments in NextGen Sequencing technologies now allow sequencing large numbers of isolates, but current analysis methods are designed for comparative analysis, and

require labour-intensive bioinformatic resources. In this study we describe the use of alignment-free algorithms for the rapid analysis of molecular phylogeny of *Campylobacter* genomes. Methods: All available genome sequences of *C. coli* (n=197) and a subset of *C. jejuni* genomes (n=95) were obtained from Genbank/EMBL, PATRIC and BIGSdb databases, and used without further preprocessing. Phylogenetic trees were created using the Neighbor-Joining Tree algorithm. Results: Validation of the analysis method using 9 *C. jejuni* and 21 *C. coli* genome sequences (Sheppard *et al.*, Mol Ecol 2013) showed clear separation of *C. jejuni* and *C. coli*. Subdivision of *C. coli* isolates was according to MLST clonal complexes and source (riparian, agricultural and clinical). Inclusion of additional *C. jejuni* and *C. coli* sequences grouped *C. jejuni* clonal complexes together (e.g. ST-48, 61 and 206), while most *C. coli* genomes clustered within clonal complex ST-828, with subclades present within this complex that await further analysis. Impact: Rapid, alignment-free analysis of whole genome sequences can be used to analyse *C. jejuni* and *C. coli* phylogeny, and uses all genomic information without requiring significant bioinformatic resources or expertise. As such it may become an important tool for analysis of outbreaks and source attribution using molecular epidemiology.

O23a - Improved methodology for the primary culture of *Helicobacter pylori* from gastric biopsies.

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Isolation and cultivation of *H. pylori* is the most time-consuming and technically difficult method for the diagnosis of this bacterium. We report here an improved method for the recovery of *H. pylori* from human gastric biopsies. We enrolled 27 patients with bleeding gastritis (mean age 36.3 years, 55.6% male) from a clinical hospital in Hanoi, Vietnam. *H. pylori* status was established by PCR of 3 specific target genes using genomic DNA extracted from antrum and corpus gastric biopsies. Antrum and corpus biopsies were plated in selective and non-selective media under micro-aerobic and anaerobic conditions. Incubation time and temperature were similar in all cases. Our results showed that blood agar and Columbia CNA plates incubated in anaerobic conditions yield significantly higher recovery rates of *H. pylori* than blood agar and Skirrow plates incubated in micro-aerobic conditions (94.1% vs. 29.4% p=0.0004). In addition, in all culture conditions tested, antrum biopsies were more likely to test positive than corpus biopsies, but the difference was not significant. Furthermore, the presence of contaminant microorganisms was not a major issue under anaerobic conditions. We concluded from this study that anaerobic conditions might provide higher recovery rates of *H. pylori* in primary cultures.

O23b - Recent increase in campylobacteriosis incidence in The Netherlands potentially related to proton-pump inhibitor use

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An unexplained increase in human campylobacteriosis was observed in the Netherlands from 2007–2011. As proton-pump-inhibitors (PPI) are an established risk factor for gastrointestinal disease, we explored a possible association. Data on annual numbers of PPI-dispenses at Dutch pharmacies were related to reported numbers of cases using negative binomial regression. Data on annual fresh and frozen chicken fillet purchases at retail were used as proxy for consumption. The former were adjusted for *Campylobacter* prevalence based on representative retail monitoring. Four age-classes were considered: 0–25, 25–50, 50–70 and ≥70. Model validity was evaluated by comparing blindly predicted case numbers for 2012 to the reported number in 2012, and to results from a case-control study of 2003. The PPI-dispenses increase exponentially from 0.2 to 0.7 doses per head of the population per year. The trend and yearly fluctuations in campylobacteriosis cases was associated with PPI-dispense (P<0.0001), age (P<0.0001), their interaction (P<0.04) and frozen chicken consumption (P=0.003). Fresh chicken consumption was not associated (*P*=0.29). The effect of PPI-dispense was largest for the youngest age and gradually decreased for older ages. The estimated counterfactual attributable proportion for PPI-dispense was 8%

in 2004 (similar to 8% from the case-control study) and increased continuously to 27% in 2011. The proportion was highest among the elderly in 2011 with ~40%. Predictions for 2012 suggested a trend break in the campylobacteriosis increase, which was confirmed by reported numbers from surveillance. Although causality is unconfirmed, this ecological study suggests a substantial impact of PPI-use on campylobacteriosis incidence in The Netherlands.

O24a - Gene-*specific* PCR analysis of *Helicobacter suis* in China

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Aims: *Helicobacter suis* (*H. suis*) is considered to be the most prevalent Gastric non-*Helicobacter pylori Helicobacter* (NHPH) species in humans. In order to obtain better insight into the prevalence of *H.suis in* Chinese *population*, gene-*specific PCR and sequencing were performed*. Methods: A total of 244 gastric mucosa samples were collected from patients complained with gastric diseases and rapid urease tests (RUT) were positive. The patients were from two hospitals in Beijing and Chongqing. The samples were grinded for extracting DNA following the protocol of QIAamp DNA Mini Kit. Primers for PCR were UreSu531FW (5'-CAC CAC CCC GGG GAA GTG ATC TTG-3') and UreSu783RV (5'-CTA CAT CAA TCA AAT GCA CGG TTT TTT CTT CG-3'). Products of PCR were detected by agarose gel electrophoresis and sequenced in Beijing Tianyi Huiyuan bioscience and technology Incorporation. Major findings: The figures of electrophoresis indicated 13 of 244 samples were positive. One of the PCR products was sequenced successfully and the DNA sequence compared with the GenBank databases revealed the similarity with the *ureA* gene of *H. suis* was 100%. Main Conclusion*: H.suis infection might be existing in Chinese population*. Impact of research: This is the first investigation about H.suis infection in Chinese population. And it will provide evidence for that H.suis would be a new important zoonotic pathogen in China.

O24b - Estimating the Financial Burden and Disease Severity of *Campylobacter* in Scotland

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Campylobacter is a burden to individuals, families and society worldwide, therefore a study was conducted to quantify the financial burden and disease severity for *Campylobacter* patients in Scotland and the UK. The objective of this study was to estimate the societal cost of human campylobacteriosis. A postal questionnaire was sent to campylobacteriosis patients in NHS Grampian to ascertain the impact of the disease on patients, families and the NHS. Data collected from the questionnaire included sequelae, duration of illness, days off work, use of NHS resources and personal out-of-pocket expenses (e.g. travel cost). The results from this study (n=253) estimated the cost per case of *Campylobacter* to be £989.75. Therefore the estimated cost of *Campylobacter* cases in Scotland in 2012 (6,312 reported cases) was approximately £6 million and the UK in 2010 (70,298 reported cases) was approximately £70 million. The average duration of illness was 10 days and it took patients an average of 4.3 days to continue with normal activities. At the time the patients completed the questionnaire 39.7% of respondents reported they were still suffering symptoms. This study has shown the calculated financial burden and severity of the infection caused by *Campylobacter* which has an impact on public health cost and directly to the patient and their family. The financial burden placed on the UK economy by human campylobacteriosis heightens the requirement for interventions to be considered to reduce *Campylobacter* cases. The outcomes from this study can help policy makers identify cost-effective interventions for *Campylobacter* from farm to fork.

O25a – *Helicobacter pylori* Infection in the Yukon Territory of Canada

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The Canadian North *Helicobacter pylori* (CAN*Help*) Working Group was invited to address health concerns related to stomach disease in the predominantly Aboriginal community of Old Crow, Yukon Territory (population ~250). Of 192

residents tested, 68% were positive for *H. pylori*. The aim of this study was to characterize the prevalence and genotype of virulence genes encoding toxins (*cagA, vacA*) and adhesins (*babA2, sabA, oipA*), with gastric histopathology scores. Gastric biopsies were collected from 63 individuals for histopathology assessment (Sidney classification), and culture for *H. pylori* for virulence genotyping by PCR and DNA sequence analysis. Of the 57 *H. pylori* positive biopsies, 32% had moderate and 65% had severe inflammation, 74% had atrophy and 35% had intestinal metaplasia. *H. pylori* was successfully cultured from 53 biopsies. The *cagA* gene was detected in 87% of isolates and of these, the EPIYA motifs were 59% ABC, 35% BC and 6% ABCC. The *vacA* gene was detected in 83% of isolates, where 64% were of s1/i1/m1 type. The *babA2* and *oipA* adhesin genes were detected in 98% and 94% of *H. pylori* isolates, respectively. The *sabA* gene was detected in 74% of *H. pylori* isolates. The high prevalence of toxin and adhesin virulence genes in *H. pylori* isolated from gastric biopsies was strongly associated with moderate-severe inflammation observed in gastric biopsy histopathology scores. The striking severity of disease and high prevalence *H. pylori* toxin and adhesin genes supports the presence of particularly virulent *H. pylori* infections present in the residents of Old Crow.

O25b - The seasonality of campylobacteriosis: are we missing something?

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The incidence rate of campylobacteriosis in humans is characterized by a striking seasonal pattern reported in all temperate countries, but is lacking consensual explanation. The objective of this study is to model the temporal characteristics of the seasonal peak of campylobacteriosis with respect to epidemiological factors such as the age group of patients and intensity of regional agriculture activity. Laboratory-confirmed cases of campylobacteriosis reported in Quebec, Canada, over a 10-year period are analyzed along with population and agricultural census data. Seasonality is assessed using regression modelling. Results will be presented. This study provides insight to factors underlying seasonality. Proper understanding of transmission pathways of Campylobacter from agriculture and other sources to humans in space and time is key for better disease control in the future.

O26a – Occurrence of *Helicobacter* suis DNA on porcine slaughterhouse carcasses.

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Introduction: *Helicobacter (H.) suis* is the most prevalent gastric non-*H. pylori Helicobacter* (NHPH) species in humans suffering from gastric disease. Recently, the presence of viable *H. suis* bacteria has been demonstrated in minced pork, suggesting that manipulation or consumption of contaminated pork is a possible route of transmission of *H. suis* bacteria. Aims: The main goal of this study was to determine the extent of porcine carcass contamination with *H. suis* in the slaughterhouse. Results: The occurrence of *H. suis* DNA was assessed on the head, mouth, mesenterial lymph nodes and palatine tonsils of pig carcasses using PCR with *ureA* gene based *H. suis*-specific primers. In total, 90 carcasses were sampled at 3 different slaughter houses. In respectively 14, 4 and 11% of all mouth swabs, head swabs and mesenterial lymph nodes *H. suis* DNA was detected. PCR products were subjected to sequence analysis of the *ureA* gene to confirm the identification of *H. suis* bacteria. In order to investigate whether *H. suis* colonization of mesenterial lymph nodes occurs during infection, pigs were experimentally infected with *H. suis* bacteria. Despite high level colonization of the porcine stomachs with the *H. suis* strain, no *H. suis* DNA was detected in the mesenterial lymph nodes. Conclusions: We demonstrate a relatively high prevalence of *H. suis* bacteria on pig carcasses, which is most likely due to contamination during the slaughter process.

O26b - *Campylobacter ureolyticus*: an Emerging Gastrointestinal Pathogen?

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The primary campylobacter-based focus of food safety and protection agencies, as well as the clinical laboratories to date, has been the detection of thermophilic species associated with human disease. Most of the routine culture methods employed in clinical laboratories favour the detection of species such as *C. jejuni* and *C coli*. However, advances in molecular detection systems highlight the unsuitability of these methods for detection of non-thermophilic *Campylobacter* spp. Recent work in our laboratory has mainly focused on the identification and characterisation of *Campylobacter ureolyticus*. A total of 7,194 faecal samples collected over a 1-year period from patients presenting with diarrhoea were screened for *Campylobacter* spp. using molecular-based methods. *C. ureolyticus* was identified in 23.8% of 349 previously genus-positive samples using a combination of 16S rRNA analysis and primers detecting the *C. ureolyticus hsp60* gene, making this species the second most common *Campylobacter* species (after *C. jejuni*) detected in faecal samples of patients presenting with gastroenteritis (at least in Southern Ireland). Moreover, using a molecular-based approach, we have also identified the presence of this species in a number of different animal sources, including unpasteurized cow's milk (12.8%). Our findings strongly suggest the possibility that *C. ureolyticus* is a zoonosis, with multiple reservoirs in nature. Isolates of *C. ureolyticus* recovered from faeces samples from patients presenting with gastroenteritis, healthy people and environmental sources show large variation both at the genetic and phenotypic levels, suggesting differences in their pathogenic potential, and prompting further investigation into their role in causing disease in humans.

O27a - Arcobacter in Humans: Intestinal Colonizer or Pathogen?

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Introduction: Arcobacters are Gram-negative, aerotolerant organisms, related to *Campylobacter.* In humans, the species *A. butzleri. A. cryaerophilus* and *A. skirrowii* have been associated with enteritis and septicemia. Limited reports on the presence of arcobacters in the human intestine have been published. In the present study, *Arcobacter* isolation was performed in a routine microbiology lab of a large secondary care hospital to collect data on human colonization and association with enteric infection. Methods: From January 2008 to December 2012, stool samples from patients with clinical suspicion of enteric disease were included. Culture for common bacterial pathogens was performed. *Arcobacter* culture was carried out inoculating 1g feces into a selective broth with subsequently plating on a selective agar. Plates were screened by dark field microscopy for typical colonies and further identified by an *Arcobacter* species-specific PCR-assay. For patients with *Arcobacter* positive stools, medical records were investigated for the definitive presence of acute enterocolitis and underlying disease. Results: From 8169 eligible samples, 5738 (70%) could be cultured for arcobacters. Within this group *Campylobacter species* were on top of the enteric bacterial pathogen list (5.6%) followed by *Salmonella* (2,35%) and toxigenic *Clostridium difficile* (1,59%). *Arcobacter* was the fourth most common isolated genus (1.22%), with almost equally isolation of *A. butzleri* (0.6%) and *A. cryaerophilus* (0.5%). Discussion: Routine *Arcobacter* recovery in humans becomes feasible. Only the need for 1g human feces limits full implementation of the technique. *Arcobacter butzleri* positives tend to be in-patients with diarrhea and an underlying disease compared to *A. cryaerophilus.*

O27b - Genomic surveillance of human *Campylobacter* isolates obtained over a one year period, from Oxfordshire, UK.

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Whole genome sequence data from 929 human *Campylobacter* isolates, obtained over a one year period (28th June 2011–27th June 2012) in Oxfordshire, UK, were used to monitor the effect of newly introduced interventions in the human food chain by comparison with seven-locus multi-locus sequence typing (MLST) data obtained for the same region since 2003. Complete seven-locus MLST data, obtained for 97.6% isolates obtained without re-testing, demonstrated that the clonal complex distribution was similar to that observed previously in Oxfordshire (2003–2009) and Scotland (2005–2006), and was consistent with chicken being a major source of human infection. Since 2003 an overall reduction in the percentage of isolates attributable to chicken (51.2%–44.2%) was noted with a concomitant increase in the proportion attributable to ruminants (39.5%–46.2%), as determined by the STRUCTURE algorithm. Relationships among isolates as determined by comparison of the 52 *Campylobacter* ribosomal subunit loci (ribosomal MLST) largely replicated the clustering observed with clonal complexes, and demonstrated the genetic diversity observed globally. The availability of whole genome sequence has facilitated further investigation of antibiotic resistance genotypes. Ciprofloxacin resistance levels were largely unchanged as compared to those reported for 2008, although some minor differences in the association between clonal complex and the antibiotic sensitivity or resistance phenotype were identified.

O28a - Whole genome-based phylogenetic clustering of *Helicobacter pylori* correlates with geographic origin and virulence factor-based typing schemes

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Introduction: Current typing of *Helicobacter pylori* is either based on multi locus sequence typing or allelic analysis of virulence factors. These methods only utilise a small part of the genome and may not represent the full genomic variation. NextGen sequencing allows for the collection of full genomic sequences but analysis of the data requires significant bioinformatic resources. Here we describe a convenient and rapid method for phylogenetic analysis of whole genome sequences, which allows for superimposing of additional information such as geographic spread and virulence factor based typing schemes. Methods: Finished and unfinished genome sequences of all 241 *H. pylori* isolates from the Genbank/EMBL and PATRIC databases were downloaded to a local PC and used without further preprocessing. Phylogenetic trees were created using the Neighbor-Joining Tree algorithm. Virulence factor typing for *cag* (presence/absence), *vacA* (s1/s2 and m1/m2) and *dupA* (presence/absence) was by a local BLAST search of the raw sequence data. Results: A phylogenetic tree constructed from the whole genome sequences matched the geographic spread of *H. pylori* isolates, with most European and North-American isolates grouping together, while South American isolates grouped with either European or East Asia/Amerindian clades. Overlaying isolates with virulence alleles revealed that *cag* and *vacA* genotypes are linked with geographic spread but *dupA* is not. Impact: Rapid, whole genome sequence-based typing of H. pylori is now accessible without the need for extensive preprocessing of the sequences or expensive bioinformatic resources.

O28b - Description of the epidemiological pattern of campylobacteriosis in Germany from 2001–2010

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Introduction: Campylobacteriosis caused by *Campylobacter* spp. is the most common notifiable bacterial gastrointestinal disease in Germany and a major problem in many other European countries as well. In contrast to other infectious diseases,

e.g., salmonellosis, the annual number of notified campylobacteriosis cases has increased in Germany and other European countries in the past years. Methods: National surveillance data from 2001 through 2010 were the basis of a detailed description of the epidemiological pattern of *Campylobacter* infections in Germany. Special focus was placed on geographical distribution and time trends of *Campylobacter* infections as well as the identification of risk groups. Results: In total, 588,308 cases of campylobacteriosis were recorded during the observed time period. The mean annual incidence increased from 67 cases/100,000 population in 2001 to 80/100,000 population in 2010. Almost 93% of the notified *Campylobacter* infections were acquired in Germany. A seasonal distribution was observed with a large peak in the summer months and a small peak in January. Incidence was highest in children ≤4 years and young adults 20–29 years of age. Especially young children living in rural regions in Germany seemed to be at high risk of *Campylobacter* infection. Impact of research: This report delivers insight into the epidemiological pattern of *Campylobacter* infections in Germany, which may be exemplary for other European countries facing problems with an increase in *Campylobacter* infections. The data show a need for enhanced and better targeted public health measures for prevention of *Campylobacter* infections.

O29a - Regulatory RNAs in the pathogenic Epsilonproteobacteria *Helicobacter pylori* and *Campylobacter jejuni*

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Genome sequencing revealed the potential proteins and a high genetic diversity of *Helicobacter pylori* and *Campylobacter jejuni*, yet little is known about transcriptome organization and post-transcriptional regulation in these major human pathogens. *Helicobacter* was even regarded as an organism without riboregulation since it lacks, like 50% of all bacteria, the RNA chaperone Hfq, a key player in small RNA (sRNA)-mediated regulation in many bacteria. However, based on a novel differential RNA-sequencing approach (dRNA-seq), we have recently defined a genome-wide map of transcriptional start sites (TSS) and identified more than 60 sRNAs in *H. pylori* strain 26695. We have now applied a comparative dRNA-seq approach to multiple *Campylobacter jejuni* isolates, to understand how transcriptome differences could contribute to phenotypic differences among closely related strains. Our study revealed that the majority of TSS is conserved among strains, but we also observed several strain-specific TSS, indicating divergent transcription and promoter usage among strains. Moreover, we identified differences in sRNA repertoires among strains which could contribute to strain-specific gene regulation. Based on our transcriptome datasets, we are now using *Helicobacter* and *Campylobacter* as new model organisms for riboregulation in virulent bacteria and bacteria that lack Hfq. We have started with the functional characterization of abundant sRNAs as well as identification and analysis of associated RNA-binding proteins. Overall, the identification and characterization of diverse sRNA candidates in *Helicobacter* and *Campylobacter* indicates that riboregulation constitutes an important layer of gene-expression control in these major human pathogens and will provide insights into novel regulatory mechanisms.

O29b - *Campylobacter* in broilers: Risk assessment as a basis for selecting a performance target

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Over two decades or so, human campylobacteriosis notifications in New Zealand gradually increased to a maximum of 384 / 100,000. As part of a comprehensive risk management response by the New Zealand Ministry of Primary Industries (MPI) and industry, a number of interventions have been introduced on either a mandatory or voluntary basis. These include a National Microbiological Database to routinely measure levels of contamination in all slaughterhouses and a regulatory performance target. The primary indicator of performance is based on a cut off of 6,000 CFU/ full bird rinsate and not more than 6 non-complying results per 15 day moving window. There has been a significant reduction in the level of contamination of broiler carcasses and human cases over the last five years. As part of a review of the performance target that had been in place for three years risk assessment was used to evaluate the likely reduction in human cases associated with increasingly stringent control measures. Estimation of different levels of risk reduction associated with further reductions

in prevalence of contaminated carcasses and the level of contamination per carcass was a key communication tool in consulting with industry on available risk management options. A new performance target will be put in place that takes into account the goal of continuous improvement in food safety and the feasibility and practicality of implementation by industry.

O30a - Mapping the *in vivo* transcriptome of *Campylobacter jejuni* using RNAseq

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Campylobacter jejuni is a major human pathogen and a leading cause of bacterially derived gastroenteritis worldwide. *C. jejuni* regulates gene expression under various environmental conditions and stresses, indicative of its ability to survive in diverse niches. Despite this ability to highly regulate gene transcription, *C. jejuni* encodes few transcription factors and the genome lacks many canonical transcriptional regulators. High throughput deep sequencing of mRNA transcripts (termed RNAseq) has been used to study the transcriptome of many different organisms including, *C. jejuni*; however, this technology has yet been applied to defining the transcriptome of *C. jejuni* during *in vivo* colonization. In addition to its use in studying ORF expression, RNAseq is a powerful tool for identifying small, non-coding regulatory RNAs, untranslated 5' regulatory elements and anti-sense transcripts. Here we report the complete transcriptome of *C. jejuni* during *in vivo* colonization of the chicken cecum and in two different *in vitro* conditions using RNAseq. Our stand-specific protocol enabled us to identify sense and antisense transcripts. Through this study we identified over 300 genes differentially regulated *in vivo* compared to *in vitro* grown cultures and have identified numerous small RNA regulators, including probable small non-coding RNAs, anti-sense transcripts and riboswitches. These latter potential regulatory elements were not identified in two prior studies using ORF-based microarrays, highlighting the power and value of the RNASeq approach. Our results illustrate the importance for continued research into how *C. jejuni* regulates gene expression during colonization of its natural host.

O30b - Microbiological criteria as a decision tool for controlling Campylobacter on broiler meat

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Campylobacter causes a substantial burden of disease in the Netherlands. Approximately 30% of all cases are attributed to preparation (cross-contamination) of broiler meat. The Dutch government advocates a process hygiene criterion (PHC) for Campylobacter on broiler meat. We evaluate the impact of a PHC using mathematical modelling, with regard to both public health (probability of illness) and producers (non-conforming batches). This is based on weekly Campylobacter count data on carcasses after cooling. There were marked differences between contamination and associated public health risks of products from different plants. Approximately 2/3 of illnesses could be prevented by implementing a PHC, with none of 5 samples per batch exceeding a critical limit of 1000 cfu/g breast skin. Approximately 1/3 of all batches produced in 2009 and 2010 would not comply with the described PHC. Note that in the model, it was assumed that non-complying batches are treated such that they pose no public health risk. This is a best-case scenario which should be replaced by more realistic scenarios. The costs of intervention to meet the PHC are expected to be considerably lower than the benefits. The costs to the Dutch poultry industry are estimated in the order of 2 million € per year, whereas the benefits to the Dutch economy are reduced costs-of-illness estimated at 9 million € per year and reduced disease burden of approximately 400 healthy life years. The benefits increase if additionally consumers of exported meat are considered, or if the PHC applies to imported broiler meat as well.

O31a - CIST: the Campylobacter *In Silico* Typing server, a resource for integrated comparative genomic analysis of *Campylobacter jejuni*

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Although molecular typing methods have traditionally been used for the characterization of bacterial pathogens, more recently, whole genome sequence (WGS) analysis has emerged as a tool that can be used to address population genetics for public health applications. WGS data can, in principle, resolve bacterial isolates that differ by a single base pair, thus providing the highest level of discriminatory power for epidemiological subtyping. We present the Campylobacter *In Silico* Typing Server, a bioinformatics resource for rapidly performing a comprehensive suite of analyses from draft genome assemblies. In addition to performing pan-genome analyses that include core genome-based phylogenetic analysis and accessory genome content distribution analysis, this resource integrates the analysis of several DNA-based genotyping schemes that include *flaA* typing, *porA* typing, Multi-Locus Sequence Typing, Comparative Genomic Fingerprinting, and AMR profiling. *In silico* genotyping results provide a link between historical typing data and WGS data, while the whole genome phylogenetic analysis provides a framework for the comparison of molecular typing methodologies. In addition, this analysis allows for the identification of genotypic clusters and the exploration of associations between specific genotypes and phenotypic/epidemiologic metadata (e.g., geospatial distribution, host, source) for the identification of group-specific genomic markers (presence/absence of specific genomic regions, and single-nucleotide polymorphisms). This platform provides efficient algorithms and pre-computed analyses for the near real-time analyses of thousands of genomic sequences for population genomics, epidemiology, and clinical studies. Collaboration between similar international initiatives will allow for a world-wide real-time surveillance and analyses network for this important pathogen.

O31b - Risk-Based Microbiological Criteria: A Tool To Control *Campylobacter*

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A microbiological criterion (MC) can be called "risk-based" if its potential impact on public health risk can be evaluated by the application of quantitative microbiological risk assessment. Recently, it has been shown that these risk-based MCs may offer a tool for *Campylobacter* control, where food safety risk managers can select the criterion that offers the best balance between cost of sanctioning and public health benefit. In Denmark, a special version of a risk-based MC has been implemented for *Campylobacter* in poultry. A so-called "case-by-case" risk assessment is applied, where the MC is expressed in terms of maximum acceptable relative risk, instead of the maximum number of samples with a concentration above a critical limit (the "traditional" approach). The case-by-case risk assessment has shown to be an effective method, resulting in a decrease in highly contaminated broiler meat batches on the market in 2007–2010. In a Nordic research project we compared the performance of the "traditional" and the "case-by-case" approach for setting risk- based MCs for *Campylobacter* in broiler meat products, by using Monte Carlo simulation models based on data from Nordic countries. In this comparison, performance is expressed as the balance between relative risk reduction and sanctioned batches for different MCs, as well as the model uncertainty. It shows that both approaches perform similarly, but the uncertainty attending the "case-by-case" approach is smaller. The data requirements for a further reduction of the uncertainty attending the risk estimates and the practical potentials for *Campylobacter* control by setting MCs are discussed.

O32a - The complete genome sequences of 65 *Campylobacter jejuni* and C. coli strains

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Campylobacter jejuni (*Cj*) and *C. coli* (*Cc*) are genetically highly diverse based on various molecular methods including MLST, microarray-based comparisons and the whole genome sequences of a few strains. *Cj* and *Cc* diversity is also exhibited by variable capsular polysaccharides (CPS) that are the major antigenic determinants of the classical Penner serotyping system. Here, we have determined the complete genome sequences of 45 *Cj* and 20 *Cc* strains representing 64 of 65 different Penner

serotypes. The *Cj* and *Cc* strains were sequenced using 454 pyrosequencing technology and contigs assembled using Newbler Assembler software. Contigs were ordered using a contig extender Perl script, Mauve software and optical maps. Gaps were computationally closed using GapResolution and Geneious programs and/or were closed by Sanger sequencing. MiSeq Illumina sequences were used to correct base calls of the final assembly. The completed *Cj* and *Cc* genomes were annotated using the RAST website, and submitted to various programs for further genomic analysis. The complete genomes revealed some significant genomic rearrangements, especially among *Cc* strains. The completed genomes allow the identification for the genetic bases of CPS variation, particularly the identification of phosphoramidate modification genes outside of the capsular biosynthetic locus. Furthermore, whole genome sequencing provides an abundance of information for comparative genome analysis including evidence of recombination of sialic acid genes from *Cj* into *Cc*. Detailed data analysis of these genomes expands our knowledge of *Cj* and *Cc* gene content and global SNP diversity, and will provide DNA signatures for the development new typing assays.

O32b - Towards a best practice for *Campylobacter* prevention at farm and house level

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The FP7 supported CamCon project deals with novel approaches to control of *Campylobacter* in primary poultry production. One of the work packages of CamCon comprises training and dissemination of project results, and aims at providing an easily accessible web-based education program for the poultry industry as one of the deliverables. Accumulated scientific evidence shows that proper biosecurity is the single most important factor to address in order to be successful in preventing introduction of *Campylobacter* to poultry flocks. However, the same evidence also points to the fact that this is difficult to achieve, and that it requires training, commitment and understanding of essential biosecurity principles from poultry farmers. In addition, an appropriate level of biosecurity at house level is a pre-requisite for reaping the full benefit of fly screens. We present results of recent work with biosecurity upgrading at Spanish broiler farms. In Spain, and in many other countries, biosecurity is mainly maintained at farm level with the purpose of preventing introduction of severe poultry diseases such as Newcastle disease while biosecurity measures at house level, which are crucial for preventing Campylobacter introduction, receive little attention. Our work has included development of posters with instruction for person entrance and exit from broiler houses, training materials for poultry veterinarians and farmers, and a check list for control of critical points of house biosecurity and management, and these will be presented. Finally, we report on the status of our work towards a Best Practice Manual for *Campylobacter* reduction at farm level.

O33a - Comparative gene-by-gene analyses of Helicobacter pylori genomes

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Helicobacter pylori colonises the gastric mucosa of approximately half the world's population and causes gastritis and peptic ulceration. H. pylori infection is the principal pathophysiological step leading to initiation of the inflammatory response and gastric cancer. However, the severity of disease and the ultimate outcome is dependent upon a complex interaction between pathogen and host cell. Chronic inflammation is understood to induce cancer by increasing reactive oxygen and nitrogen species and subsequent DNA damage. This could result from infection with any H. pylori stain. However, H. pylori populations are highly structured with numerous genotypes existing together in a single patient and these strains can have different disease causing potential. The advent of high-throughput whole genome sequencing provides a powerful tool to compare epidemiological, physiological and genetic variation using data from hundreds or thousands of bacterial genomes at the same time. In this work, we used 244 publicly available genomes of isolates within the genus Helicobacter and present a description of basic population genomics analyses, using a gene-by-gene comparative approach, centred on a publicly accessible database platform. We describe the use of a gene-by-gene approach, and its functional capabilities. Furthermore, we performed comparative analyses and observed variations in the core and accessory genome reflective of the phylogenetic history within the Helicobacter genus. The freely available gene-by-gene analysis pipeline that we present provides a rapid tool for epidemiological and evolutionary analysis of Helicobacter and offers the potential for further increasing the use of whole genome sequencing in clinical microbiology.

O33b - Monitoring of campylobacters in UK poultry slaughter batches and carcasses and collection of information from primary production and processing for risk factor elucidation

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Aims: To monitor the numbers of *Campylobacter* on chicken broiler carcasses and use multi-factorial statistical analyses to identify farming or processing practices that are associated with lowered numbers of campylobacters on carcasses. Methods: Analyse industry-donated, quantitative *Campylobacter* test results from neck skin samples collected and tested in a standardised manner from post-chill chicken carcasses. In addition, for each set of test results, a series of five questionnaires were requested to be completed which covered the farm and processing plant infrastructures, the conditions experienced during the growing of the birds on farm and at the time/on the day of processing. Major finding: Based on an incomplete dataset of several thousand *Campylobacter* test results and over 1500 completed processing questionnaires, there are process properties such as hall volume, operating temperature and carcass washing which can significantly influence numbers of campylobacters on carcasses. Main Conclusion: Preliminary analyses suggests that numbers of campylobacters on chicken carcasses processed from campylobacter-colonised birds can be significantly reduced by the practices undertaken at some processing stages. Impact: Presumptive processing methods which result in lower numbers of campylobacters on chicken carcasses have been identified from an analyses of an incomplete dataset. Since these practices are used in commercial processes, they are practical to implement and economically achievable.

O34a - *Campylobacter Jejuni* Peptidoglycan-Modifying Enzymes: New Players Controlling Helical Shape and Pathogenic Properties

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The corkscrew shape of *Campylobacter jejuni* is a hallmark feature of its morphology, and had been hypothesized as important for virulence-related attributes. We recently published the first factor required to maintain shape, Pgp1. Pgp1 is a novel peptidoglycan carboxypeptidase cleaving monomeric tripeptides to dipeptides. *pgp1* deletion resulted in straight morphology and key phenotypic changes that affect all aspects of *C. jejuni's* lifecycle: transmission, colonization, pathogenesis. Using both *in silico* and mutant screening approaches, we have now identified a number of new enzymes that also impact peptidoglycan, are required for maintenance of morphology, and are important for numerous *C. jejuni* lifecycle attributes. For instance, biochemical and mutant analyses showed that one, Pgp2, is a carboxypeptidase that cleaves monomeric and dimeric tetrapeptides to tripeptides, creating the Pgp1 substrate. Like *pgp1*, loss of *pgp2* resulted in a completely straight morphology, motility and biofilm defects, and diminished chick colonization. Despite having a similar shape, differences in peptidoglycan composition between the two mutants resulted in different pathogenic properties: while peptidoglycan from the *pgp1* mutant hyperstimulated the intracellular human innate immune receptor hNod1, peptidoglycan from the *pgp2* mutant completely abrogated the hNod1 response. On going molecular, genetic, and biochemical analyses of these and other peptidoglycan-modifying enzymes are continuing to unravel the heretofore unstudied *C. jejuni* peptidoglycan biosynthesis and remodelling programs, and the effect of these modifications on morphology and other pathogenic characteristics. We are also further identifying significant differences between *C. jejuni* peptidoglycan dynamics and those of well-studied models such as *E. coli* and *Bacillus* spp.

O34b - The *Campylobacter jejuni* protein glycosylation pathway-derived heptasaccharide is an effective chicken vaccine

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Campylobacter jejuni is a significant foodborne pathogen and the most common cause of human gastroenteritis. Since infection occurs predominantly through the handling and consumption of poultry, elimination of *C. jejuni* from chickens

would significantly reduce the incidence of infection in humans, however, steadily increasing antibiotic resistance makes its control challenging. In this study, we target the highly immunogenic *C. jejuni* heptasaccharide produced by the protein glycosylation machinery. The fact that the underlying biosynthetic pathway can be transferred to *E. coli* opens the possibility for heterologous expression of various glycoconjugates that can be used in vaccine formulations. Using a 35 day immunization and challenge protocol, we tested the efficacy of different *C. jejuni* heptasaccharide-based glycoconjugates. Vaccination with a glycoprotein conjugate resulted in up to 5-log reduction of Campylobacter colonization compared to the control group, independent on injection site or dosage. We also expressed the heptasaccharide on the surface of attenuated *E. coli* cells. Oral administration of the inactivated strain led to a 4-log reduction in *C. jejuni* colonization while vaccination with the live *E. coli* strain reduced colonization by 8-logs. Also, the heptasaccharide-specific IgY titre was highest in these vaccinated birds and the *E. coli* vaccine strain was cleared minimizing the risk of population shifts in the chicken gut flora. We propose that *E. coli* expressing the *C. jejuni*-heptasaccharide is an effective and low-cost live vaccine significantly reducing *C. jejuni* chicken colonization and therefore its entry into the food chain.

O35a - Defining the role and regulon of the C. *jejuni* peroxide stress regulator (PerR)

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Campylobacter jejuni is able to survive food preparation and the oxygen-exposed route from its avian host, entering into the human food chain where it proliferates. PerR has previously been identified as one of the main regulatory proteins involved in the protective response of *C. jejuni* to oxidative stress. *C. jejuni* PerR regulates gene expression in a metal-dependent manner, controlling transcription of a set of peroxidases. Whilst targets of PerR have been identified previously by microarray analyses, there is little consensus of the PerR regulon between comparative studies. Here we have inactivated *C. jejuni perR* and characterised the *perR* regulon using RNA sequencing to identify target promoters, proteomics (2D gel electrophoresis) and gel shift assays to confirm direct regulation. By combining these technologies we have been able to focus and hone in on the core members of the PerR regulon. PerR inactivation is unique amongst *C. jejuni* biology, as it is currently (to our knowledge) the only gene deletion that provides a competitive advantage over the wild type *C. jejuni* strain, as a *C. jejuni perR* mutant displayed increased aerotolerance and survival against exposure oxidative stress compared to the wild type strain. A *C. jejuni perR* mutant did not have any defect in in vitro growth, motility or killing in the *Galleria mellonella* virulence model. Further investigations are required into the role of PerR in *C. jejuni* to decipher why PerR regulation persists despite the beneficial nature of the *perR* gene deletion for survival.

O35b - The creation of an anti-C*ampylobacter jejuni* multivalent vaccine for humans.

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Campylobacter jejuni is among the primary causes of diarrhea-related illnesses worldwide and an effective vaccine would be welcomed. *C. jejuni* serotype complexes are defined based on the covalent structures of the corresponding capsule polysaccharides (CPSs). Among other structural aspects, the expression of heptoses of unusual configuration and O-methyl-phosphoramidate substitution patterns are key features in dictating serospecificity. *C. jejuni* strains sharing these structural characteristics may be part of the same serotype complex. We have discovered the chemical structures of CPSs belonging to the most prevalent *C. jejuni* serotype complexes, and have used them to create CPS-conjugate vaccines. Here, we will present the fine synthesis and immunogenicity of CPS-conjugate vaccines against several *C. jejuni* serotype complexes, and the effectiveness of a CPS-based vaccine to protect against heterologous challenge within a serotype complex in a monkey model. Collectively, the data strongly suggests that a multivalent CPS-based vaccine can prevent C. jejuni diearrhea in humans.

O36a - Enhancing Aerobic Growth of Campylobacter in Media Supplemented with Organic Acids

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The effect of agar and sodium bicarbonate ($NaHCO_3$) concentration on aerobic growth of *Campylobacter* was determined. A fumarate-pyruvate medium was supplemented with 0.0 to 0.2% agar and inoculated with *Campylobacter coli*, *Campylobacter fetus*, or *Campylobacter jejuni*. Portions of the inoculated media were transferred to wells of a honeycomb plate of a Bioscreen Microbiology Reader. The optical density (OD) of cultures was measured during aerobic incubation at 37C for 72 h. Next, fumarate-pyruvate media containing 0.15% agar was supplemented with 0.00 to 0.10% $NaHCO_3$ and inoculated with *Campylobacter* then growth was measured as previously described. Additionally, experiments were conducted to compare the number of *Campylobacter* recovered from media supplemented with 0.15% agar and 0.05 % $NaHCO_3$ and incubated aerobically or microaerophilically for 72 h at 37C. Results of experiments indicated that the OD of cultures of all *Campylobacter* isolates was significantly higher when grown in fumarate-pyruvate broth media supplemented with agar. Furthermore, addition of $NaHCO_3$ to the medium supplemented with agar produced a significant increase in the OD of most isolates. Also, after 72 of incubation there was a 5 to 6 log increase in *Campylobacter* recovered from inoculated media containing 0.15% agar and 0.05% $NaHCO_3$, and there was no significant difference in the number of CFU recovered from media incubated aerobically or microaerophilically. Findings indicate that supplementing fumarate-pyruvate broth medium with agar and $NaHCO_3$ produces significant increases aerobic growth of *Campylobacter*. This medium might provide a less expensive, simplified alternative to current procedures for culturing *Campylobacter* under microaerophilic conditions.

O36b - Immunogenicity of a *Campylobacter jejuni* flagellin-based subunit vaccine in chickens

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Contaminated poultry meat products are considered as the major source of human cases of campylobacteriosis. Several strategies are considered to decrease the number of *Campylobacter* in the intestinal tract of chickens what in turn would reduce infections in humans. One of these strategies is vaccination. Our aim was to develop a flagellin-based subunit vaccine with intrinsic adjuvant activity. The immunostimulatory properties make bacterial flagellin a potent vaccine adjuvant, however *C. jejuni* flagellin is unable to activate TLR5, in contrast to the flagellin of most other bacterial species. We constructed a chimeric NHC flagellin that contains fragments from *Salmonella enteritidis* flagellin which is able to potently activate TLR5. To assess the potential of this flagellin-based vaccine, *in ovo* vaccination was performed at day 18.5 incubation of the egg. Additionally, one group of chickens was immunized with a *C. jejuni* 81116 total cell lysate. Serum samples were taken at 11 and 15 days after hatching to assess the specific immune response by ELISA. *In ovo* vaccination demonstrated the successful generation of IgG antibodies against flagellin-based subunit vaccine and total cell lysate of *C. jejuni* 81116. Our results indicate that *in ovo* vaccination with a protein subunit vaccine is an effective way to generate a specific antibody response against *C. jejuni*.

O37a - Modelling Bacterial Persistence in Campylobacter jejuni.

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The observation that a small fraction of a bacterial population survives antibiotic treatment was first made around 70 years ago. However since then very little interest has been paid to this phenomenon until quite recently. These persister cells do not pass on their tolerance to their progeny and have been identified as a means by which bacteria may establish chronic infections, which are refractory to treatment with antibiotics. The mechanisms of bacterial persistence are now a major area of research interest. We have confirmed that under certain antibiotic stresses campylobacter jejuni has a persistent subpopulation which survives up to 24h of 100xMIC exposure to certain antibiotics. We propose that without an understanding

of the mechanisms underlying this persistence phenomenon, our ability to treat against the threat of campylobacter jejuni contamination in the food chain will be greatly impaired. One of the prevailing ideas in the field today is that bacterial cells switch stochastically between a normal susceptible state and a phenotypically persistent state which is tolerant to antibiotics. However there are identified features of persister cells in different bacterial strains which this model is not equipped to account for. We hypothesise that a model incorporating the effect of accumulating damage which can reproduce many of these observed phenomenon. We also aim to use the model as a means of predicting the evolution of persister frequencies in fluctuating environments and will report on both insilico and wet lab evolutionary experiments of fluctuating environments.

O37b - Use of a Recombinant Attenuated Salmonella Typhimurium Vaccine Vector for the Reduction of *Campylobacter jejuni* in Broiler Chickens

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Campylobacter jejuni is a leading cause of gastroenteritis globally, responsible for tremendous physical and financial burden in both developing and developed nations. Within the U.S., *Campylobacter* causes an estimated 1.3 million cases of campylobacteriosis annually, resulting in health care costs of $0.8–5.6 billion per year. The risk factor most closely associated with infection is consumption of poultry, as the organism is commensal in birds and prevalence remains high in commercial flocks despite current intervention strategies. Furthermore, risk assessment indicates a 2-log reduction of *Campylobacter* on chickens would result in a 30-fold reduction of the incidence of campylobacteriosis associated with chicken meals. The authors have developed a Recombinant Attenuated *Salmonella* Vaccine (RASV) vector expressing various *Campylobacter* proteins previously shown to be highly expressed in poultry for the reduction of *Campylobacter* in chicken feces, thus reducing eventual contamination of chicken meat. Cornish X Rock broilers vaccinated with the RASV expressing *C. jejuni* protein Laj1 at 10 and 16 days of age and challenged with the homologous human clinical isolate *C. jejuni* NCTC 11168 at 26 days were euthanized at 36 days of age and assayed for levels of *Campylobacter* in cecal contents. Additional trials were conducted using the same vaccine in a water-based delivery strategy. *Campylobacter* colonization was reduced in vaccinated groups by approximately 2.5–3 logs as compared to empty vector and positive control groups. Additional trials investigating protection against heterologous *Campylobacter* strains and utilizing multiple antigens have also yielded promising preliminary results.

O38a - Two small non-coding RNAs post-transcriptionally control flagellar gene expression in *Campylobacter jejuni*

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The bacterial pathogen *Campylobacter jejuni* is the leading cause of foodborne gastroenteritis in the developed world. Non-coding RNAs (ncRNAs) are important regulators of prokaryotic biological and virulence processes. Two paralogous small ncRNAs (less than 50 nucleotides), CjNC1 and CjNC4, have been identified in the *C. jejuni* transcriptome, and are predicted to target sigma54-dependent, flagellar genes. The aim of this study was to characterise the function of CjNC1 and CjNC4 in *C. jejuni* NCTC 11168 flagellar regulation. CjNC1 and CjNC4 expression was markedly decreased in *C. jejuni* sigma28-deficient mutants, as shown by RT-PCR and Northern hybridisation. Phenotypic analysis of *CjNC1*/*CjNC4* inactivation and over-expression mutants showed little differences in motility, biofilm formation or invasion of intestinal epithelial cells. Microarray analysis showed no clear differences in gene expression between *CjNC1*/*CjNC4* inactivation and over-expression mutants compared to the wild-type strain. However, using a plasmid-based GFP reporter system in *E. coli*, CjNC1 and CjNC4 post-transcriptionally regulated expression of the predicted sigma54-dependent genes *Cj0428* (*hypothetical*), *Cj1729c* (*flgE2*) and *Cj1650* (*hypothetical*). Moreover, mutating the ncRNAs disrupted GFP regulation indicating that ncRNA-target binding via complementary base-pairing is required for regulation. In summary, CjNC1 and CjNC4 transcription is dependent on the flagellar sigma factor, sigma28, and post-transcriptionally controls expression of sigma54-dependent, flagellar genes in *C. jejuni*. This is the first description of small RNA-mediated gene regulation in *Campylobacter* species, although its biological significance is as yet unknown.

O38b - Development of an adenovirus vectored vaccine for the prevention of colonization of poultry by *Campylobacter*

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Colonization of poultry by Campylobacter jejuni is a significant food safety risk. C. jejuni, a harmless commensal in poultry, causes disease once they are transferred to humans, usually via handling or consumption of insufficiently poultry products. Vaccination to prevent colonization is one strategy to reduce the risk. In poultry, delivery of vaccines, especially to mucosal surfaces, presents a challenge as large numbers of animals must be vaccinated in a manner that is cost effective. The use of adenovirus vectors, for the delivery of poultry vaccines, appears promising and needs further investigation. In other species, adenovirus vectored vaccines have produced a strong mucosal immune response. We believe that a mucosal immune response is necessary to prevent colonization of poultry by Campylobacter as previous work at VIDO has shown that birds having high levels of circulating antibodies are not protected from infection by these bacteria. We will determine if adenovirus vectored vaccines are effective in producing this response. We have generated recombinant adenoviruses expressing several surface genes of *C. jejuni*. These viruses have been delivered by in ovo vaccination, a route of delivery widely used in the poultry industry, provides an automated system for vaccination 2 to 3 days prior to hatch. The birds were orally challenged at approximately three weeks of age to determine the efficacy of the vaccine.

O39 - New Insights into Helicobacter pylori Pathogenesis

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The attributable risk for gastric cancer conferred by Helicobacter pylori is approximately 75%. However, only a fraction of colonized persons ever develop neoplasia, and disease risk involves well-choreographed interactions between pathogen and host, which are dependent upon strain-specific bacterial factors, host genotypic traits, and/or environmental conditions. These observations, in conjunction with evidence that carriage of certain strains is inversely related to esophageal adenocarcinoma and atopic diseases, underscore the importance of understanding mechanisms that regulate biological interactions of H. pylori with their hosts that promote carcinogenesis. H. pylori strains are extremely diverse, freely recombining as panmictic populations. One strain-specific virulence determinant that augments the risk for gastric cancer is the cag pathogenicity island, a type 4 secretion system that injects the bacterial oncoprotein CagA into host epithelial cells. Host polymorphisms within genes that regulate immunity and oncogenesis also heighten the risk for gastric cancer, in conjunction with H. pylori strain-specific constituents. Further, environmental conditions such as iron deficiency and high salt intake augment the expression and deployment of H. pylori virulence constituents that lower the threshold for disease. Delineation of bacterial, host, and environmental mediators that augment gastric cancer risk has profound ramifications for both physicians and biomedical researchers as such findings will not only focus prevention approaches that target H. pylori-infected human populations at increased risk for stomach cancer, but will also provide mechanistic insights into inflammatory carcinomas that develop beyond the gastric niche.

O40 - The FSA's Campylobacter Reduction Strategy

Prof Charles Milne
Food Standards Agency, Aberdeen, UK

The Food Standards Agency (FSA) is a non-ministerial government department, governed by a Board appointed to act in the public interest, responsible for food safety and hygiene across the UK. It is estimated that each year in the UK almost a million people suffer a foodborne illness, 25,000 require hospital treatment and around 400 die, with a total annual cost of £1.5 billion. The FSA's Strategy for 2010–2015 highlights the reduction of foodborne disease as a continuing priority, ensuring that food produced and sold in the UK is safe to eat. The FSA's Foodborne Disease Strategy targets those foodborne pathogens responsible for the greatest disease burden and whose effective control can offer the greatest public health gains. Campylobacter remains the most commonly reported bacterial cause of infectious intestinal disease (IID) in the UK. Evidence indicates that 65% of chickens at retail sale in the UK are contaminated with Campylobacter and that up to 80% of clinical infections may be attributed to chicken. The FSA is working in partnership with producers and retailers through a Joint Government/

Industry working group to achieve an agreed target to reduce levels of Campylobacter contamination of UK chicken by 2015. A programme of research coordinated with other funders is underway to support this and identify and develop effective interventions across the food chain to control Campylobacter. This presentation provides an overview of the FSA's strategy for reducing Campylobacter and the contribution of research in the development of effective intervention strategies.

O41 - Prevention of gastric cancer by eradication of H. pylori. Is it time for mass eradication?

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Helicobacter pylori infection is the main infection leading to cancer worldwide. Easy tools are currently available to make a diagnosis and to eradicate the bacteria. Having a healthy stomach would also decrease the burden of other diseases such as peptic ulcer disease, improve non–ulcer dyspepsia in a minority of patients, and possibly avoid autoimmune and cardiovascular related diseases. Otherwise we do not yet have definite proof of the impact of infection on gastric cancer given the existence of a 'point of no return' of the injured mucosa. Nevertheless, mouse studies have shown that eradication 2 and 6 months post infection (PI), is totally protective, while eradication 12 months PI decreased the cancer rate by 75%. In humans, the Shandong intervention trial (China) showed an OR of 0.16 [95% CI 0.38–0.96%] in favour of eradication *vs.* placebo, and a population-based study of *H. pylori* eradication on an island in Taiwan led to a 25% decrease in the incidence of gastric cancer after 5 years. We can anticipate that such a strategy will increase the rate of resistance in many different bacteria including *H. pylori* and will be costly for a long term benefit in addition to the usual ethical concerns. Therefore, before the implementation of a mass programme, tests should be carried out and it is likely that the cost benefit will vary considerably in different regions throughout the world.

O42a - MBiT: Molecular typing of *Campylobacter jejuni and C. coli* in less than six hours and under €10!

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Fast and inexpensive subtyping is now a reality. With the aim of making subtyping of all *Campylobacter jejuni* isolates a possibility for laboratories around the World, our group previously developed a 18-target PCR Binary Typing (P-BIT) system for *C. jejuni* based on pathogenicity- or survival-associated genes. P-BIT requires no specialised laboratory equipment or software. We have now converted the P-BIT to a Multiplex Ligation-dependent Probe Amplification (MLPA) format that allows all 18 products to be examined in a single reaction and discriminated using chip-based electrophoresis systems such as Shimadzu's MultiNA as well as capillary electrophoresis. It is also possible, by undertaking the assay in two tubes, to achieve acceptable results using gel electrophoresis. This new MBiT assay was tested on 244 isolates, including 139 from New Zealand, 92 from Belgium, 11 from Denmark, 2 from USA and 1 from the UK. All strains were also examined with MLST: MBiT was more discriminatory (Simpson's Diversity Index [DI] = 0.986) than MLST (DI = 0.979). MBiT's discriminatory potential is comparable to studies that have used *Sma*I-derived PFGE for subtyping. MBiT is possible using crude DNA extracts and PFGE plugs in addition to Chelex® and DNeasy extractions. When applied to a recent waterborne outbreak of *C. coli*, results consistent with PFGE were available several days before the reference method and resulted in additional cases being included in the outbreak. MBiT results are possible within 6 h at a less than €10 for consumables making it a rapid and inexpensive subtyping system.

O42b - The call of the wild - lessons from environmental campylobacters

Jonas Waldenström
Linnaeus University, Kalmar, Sweden

Few gastrointestinal pathogens have received so much attention from researchers as *Campylobacter jejuni*. Literally thousands of articles have been published on various aspects of the biology and epidemiology of *C. jejuni* over the last 30 years. Although significant progress has been made, we still haven't pinned down some of the most fundamental properties of *C. jejuni* biology. For instance, why is there such huge strain diversity? What determines host associations? Which properties make certain strains more successful than others in colonizing and maintaining presence in different animal niches, and the environment? In this talk I will argue that it is time to really start to investigate non-food animal *C. jejuni* strains, as they may very well be the key for our understanding of what makes *C. jejuni* the pathogen it is today. I will use data from our on going studies on C. jejuni in wild birds, but also sum-up some trends from the last three decades.

O43a - A novel Immuno-line Assay enables highly specific and sensitive serologic diagnosis of *H. pylori* Infection and predicts histopathologic progression

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H. pylori infects half of the world's population, but only a minority of infected individuals develop associated diseases. To date, it is not possible to identify patients with increased risk for disease. *H. pylori* virulence factors have been associated with disease development, but direct assessment of virulence factors requires invasive methods. Our study aimed at the development of a non-invasive, serologic test to detect immune responses against important *H. pylori* virulence factors. This immuno-line assay system (recomLine^{R}) is based on recombinant proteins produced in *E. coli*, which function as antigens and are applied to a solid phase. CagA, VacA, GroEL, gGT, HcpC and UreA were expressed in *E. coli*, purified and immobilized to nitrocellulose membranes to detect immune responses in patient's sera. For the validation of the line blot a cohort of 500 patients was screened, of which 290 (58.0 %) were *H. pylori* negative and 210 (42.0 %) were positive in histology. The assay showed a sensitivity and specificity of 97.6 % and 96.2 % respectively compared to histology. In direct comparison to the lysate blot and ELISA, the *recom*Line had increased discriminatory power. We also could show that the recomLine^{R} score correlates significantly with the degree of inflammation and the degree of activity in infected patients, and correlates with the risk of progression from atrophy to dysplasia in a high risk population. Taken together, the *recom*Line provides a valuable tool for the diagnosis of *H. pylori* infection, and might help to select patients for eradication therapy.

O43b - Real-Time Surveillance of *Campylobacter* Linked to Detection in Environmental Waters and Wastewater

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Aims: Detection and genetic diversity assessment of *Campylobacter* in environmental settings needs intensified attention in order to acquire a more complete picture of the pathogen's epidemiology. Here, characterisation of clinical and water-related *Campylobacter* isolates was performed over 2 years in Luxembourg, and interconnection of genotypes from both origins was investigated. Methods: Clinical samples were collected in the entire country for best-possible representativeness of human *Campylobacter* diversity. *Campylobacter* detection in surface water and wastewater was done using an optimised passive-filtration method. The genotyping strategy was based on the routine MLST scheme plus the porA and gyrA markers for enhanced discrimination power. Major findings: 1,300 human isolates and 900 environmental isolates were collected. Infection mostly followed isolated or micro-epidemic patterns. Seasonal influences and age/gender divergences were observed, with higher incidence among small children and females between 20–35 years old. Among the water and wastewater isolates, 363 genotypes were described, 20% of them being new MLST profiles. About 60% of treated sewage effluents were

Campylobacter-positive, thus contributing to its dissemination in the environment. Importantly, 62 of the water-related genotypes were found in 340 human cases. Conclusions: This study highlighted the immense diversity and the multiple shared genotypes of *Campylobacter* among the population and in water-bodies. Transmission from the original animal reservoir to the water cycle and contact to humans still remains unclear, and further research efforts are needed. Impact of the research: These findings contributed to alert the Luxembourg health authorities, raising *Campylobacter*-related public health issues to a "national-priority" for deciding on preventive measures and increasing population awareness.

O44a - Optimization of atmospheric gas mixtures for cultivation of *Campylobacter jejuni* and *C. coli*

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Exact atmospheric gas requirements for optimal growth of *Campylobacter* spp. have not previously been defined. It is accepted that these microaerophiles require both an oxygen concentration and a carbon dioxide concentration in the range 5% to 10% and an oxygen concentration at the lower end of this range is usually preferred. Thus, the incubation atmosphere most commonly used consists of 5% O_2, 10% CO_2 and 85% N_2. We used a variable atmosphere workstation to investigate the optimal gas mixture for cultivating *C. jejuni* and *C. coli* strains of human and bovine origin. The instrument achieves accurate gas mixing via an integrated control system to rapidly create and maintain the selected parameters of gas concentration and humidity. All experiments were conducted at 37ºC. Concentrations of O_2 and CO_2 were independently varied over the range 5% to 10%. The degree of growth exhibited by each *Campylobacter* strain on Columbia Blood Agar after 72 h incubation in each test atmosphere was assessed on the basis of colony diameter. Our results demonstrated that an atmosphere containing low concentrations (5%) of both O_2 and CO_2 produced poor growth of all strains. However, optimal growth was achieved when the concentration of either O_2, CO_2 or both gases was increased to 10%. Thus, further research is needed to elucidate the precise role of O_2 and CO_2 in the growth of *Campylobacter* species.

O44b - Combining source attribution and epidemiological data: a tool for investigating source-associated risk factors for human campylobacteriosis

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A combined case-control and Multilocus Sequence Typing (MLST)-based source attribution analysis was performed to investigate risk factors at the point of exposure for human campylobacteriosis of chicken, ruminant (cattle and sheep), environmental (water, sand and wild bird), pet (dog and cat) and exotic (travel-related) origin. *Campylobacter jejuni* and *C. coli* strains from 737 human cases in The Netherlands included in a case-control study comprising 3119 frequency-matched controls were typed using MLST. The Asymmetric Island model for source attribution was used to estimate the probability for the sequence types (STs) found in human cases to originate from each of the considered reservoirs. Cases were then split according to their attributed reservoirs. Reservoir-specific risk factors were investigated using logistic regression analysis. Most cases (~87%) were attributed to chicken and cattle. Chicken consumption increased the risk for chicken-attributed infections, whereas consuming beef and pork was protective. Animal contact, barbecuing in non-urban areas, tripe consumption, and never/seldom chicken consumption were risk factors for ruminant-attributed infections. Game consumption and swimming in household swimming pools in springtime increased the risk for environment-attributed infections. Dog ownership increased the risk for environment- and pet-attributed infections. Person-to-person contacts around holiday periods were risk factors for domestic infections with exotic STs, putatively introduced by returning travellers. We concluded that individuals acquiring campylobacteriosis from different reservoirs have different associated risk factors, the identification and characterization of which allow public health messages to be targeted more effectively. The outcome of classical case-control studies can be enhanced by incorporating source attribution data.

O45a - Comparison of six culture protocols for isolation of *Campylobacter* spp. from faecal and meat samples

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Introduction: Campylobacteriosis is most commonly associated with *C. jejuni* and *C. coli* infection. Other *Campylobacter* spp. have been implicated as pathogens and are considered to be underestimated mainly due to the widespread use of culture conditions optimised for *C. jejuni/coli*. Many culture protocols for the variety of sample matrices screened for *Campylobacter* spp. exist and this study aimed to compare six culture protocols applied to both meat and faeces. Methods: One freshly voided canine faecal and one frozen/thawed home-kill meat sample were taken per farm from the Manawatu region, New Zealand. Samples were refrigerated and processed within 6 hours of collection. Six culture protocols were employed varying by temperature (37 and 42°C), atmosphere (microaerobic and H_2-enriched), inclusion and/or type of enrichment broth, and selective method (CAT and mCCD agars and filtration procedure) used. Samples were incubated for 6 days. Genus and species confirmation was performed by PCR using standard primers. Results: From the 50 farms surveyed, the overall prevalence of *Campylobacter* spp. by all methods combined was 62% and 12% in dog faeces and meat, respectively. The prevalence rates by each of the methods individually ranged from 4 to 55% (with significant differences) in faeces and 2 to 4% in meat. The methods also differed by the range of species detected. A significantly higher isolation rate of *C. jejuni* and *C. upsaliensis* was associated with the use of CAT agar and/or 37°C. The selection of culture protocols are of clinical and epidemiological relevance especially for investigation of emerging *Campylobacter* spp.

O45b - Prevalence, host association, and diversity of *Campylobacter, Arcobacter*, and *Helicobacter* in reptiles and amphibians

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Campylobacter, *Arcobacter*, and *Helicobacter* (CAH) species occupy a broad vertebrate host range and have been isolated from birds, mammals, and reptiles. Multiple studies have focused on the prevalence of these *Epsilonproteobacteria* genera in many avian and mammalian species. However, little focus has been on presence in reptiles and amphibians, and their potential zoonotic and pathogenic role. In a broad-scale study, CAH prevalence, host association, and diversity were determined for a large variety of reptiles and amphibians. From 2011 to 2012, 438 cloacal swabs and fecal samples originating from 410 predominantly captive-held reptiles and amphibians were screened for CAH. Samples were cultured on selective media and screened phenotypically for CAH. Using a tiered approach of AFLP, *atpA* and 16S rDNA sequencing, 409 isolates were characterized up to the (sub)specific level. Overall CAH prevalence was 15.5%. Turtles showed highest prevalence of CAH (29%), followed by lizards (16%) and snakes (3%). One CAH species was isolated from amphibians. Most commonly isolated species were *Arcobacter butzleri*, *Arcobacter skirrowii*, and two novel *Campylobacter* (sub)species: *Campylobacter fetus testudinum* subsp. nov. and *Campylobacter iguaniorum* sp. nov.. The latter has thus far only been isolated from reptiles. Undescribed *Helicobacter* species were isolated from lizards and turtles. This study shows that reptiles carry various CAH species, including several novel species. A novel *Campylobacter* species, *Campylobacter iguaniorum* sp. nov., seems to be confined to reptilian hosts.

O46a - Optimization and validation of a *Campylobacter* genera-specific qPCR assay: A molecular tool to test anecdotal *Campylobacter* species prevalence within environmental samples

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Introduction: Culture-based investigations of *Campylobacter* species prevalence in particular samples (e.g. poultry is 95% *C. jejuni*, 5% *C. coli*, swine is 95% *C. coli*, 5% *C. jejuni*) often only used limited numbers of isolates to determine these ratios.

There is a general lack of research using molecular tools to enumerate the *Campylobacter* genera as a whole or to test these anecdotal environmental ratios of *Campylobacter* species. Methods: A *Campylobacter* genera qPCR primer set was optimized against a panel of 13 *Campylobacter* species and 9 non-*Campylobacter* e-proteobacteria both in terms of specificity and limit of detection (10^7 – 10^1 cfu/qPCR reaction). To determine *Campylobacter* species ratios, qPCR efficiencies and limits of detection for the *Campylobacter* genera qPCR were compared to *C. jejuni*, *C. coli*, and *C. lari* specific qPCR assays, and predefined ratios of cultures from these three *Campylobacter* species were created and quantified using all four qPCR assays (1 genera- and 3 species-specific assays). Results: The *Campylobacter* genera-specific qPCR was specific for all *Campylobacter* species over the entire range of concentrations tested, and exhibited similar efficiencies to the *Campylobacter* species-specific qPCR assays. The use of this genera-specific qPCR assay in combination with *Campylobacter* species –specific qPCR assays allowed for the accurate determination of species ratios within cultural mixtures. Impact of Research: Largely anecdotal species ratios within environmental samples can now be molecularly tested using a combination of this genera-specific and species-specific qPCR assays, which can greatly expand our knowledge of *Campylobacter* diversity in the environment.

O46b - Quantitative estimation of *Campylobacter jejuni* survival in house flies at 20°C and 42°C after inoculation with 3×103 CFU.

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Flies have been reported as transmitters of *Campylobacter* into broiler houses. However, it is uncertain if flies serve as passive carriers or if colonization of the fly gut is feasible under favorable temperatures. This study aimed to follow the *Campylobacter* numbers in flies over time at two temperature regimes. Methods: A total of 35 newly emerged house flies of a laboratory breed were inoculated on their proboscis with 1 µl of a *Campylobacter jejuni* suspension containing 3.5×10^3 CFU. The inoculated flies were held dark for 0 h (positive controls), 2, 4 and 8 h at 20°C and 42°C (5 replicates per treatment) before enumeration of *C. jejuni* by plate spreading of 10-fold dilution series of homogenized single flies onto Abeyta-Hunt-Bark agar. Results: Two hours post-inoculation, the *C. jejuni* numbers in flies were similar at 20°C (2.5×10^3 CFU) and at 42°C (1.4×10^3 CFU). All flies held at 20°C were *C. jejuni* positive after 4 h (2.2×10^2 CFU) and 8 h (1.7×10^2 CFU). At 42°C, the *C. jejuni* numbers were reduced to 4.4×10^1 CFU (2 positive flies) after 4 h and to zero after 8 h. In comparison, *C. jejuni* (3.5×10^3) inoculated into Brain Heart Infusion Broth in a microaerobic atmosphere at 42°C increased to 1.1×10^6 CFU after 8 h incubation. Conclusion: The reduction in *C. jejuni* numbers over time indicated that the house flies served merely as passive carriers and that *C. jejuni* did not colonize the fly gut even at their optimum growth temperature (42°C).

O47a - Crucial parameters for a reliable quantification of viable *Campylobacter* by real-time PCR

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The importance of cultivation-independent quantification of *Campylobacter* on chicken carcass skin is obvious. However, there is much confusion about what exactly is detected by real-time PCR methods in combination with DNA intercalator dyes, penetrating dead bacterial cells, thereby inactivating their DNA for detection. We found that the method has multiple pitfalls to be considered, resulting in variation of the results if crucial parameters were not kept constant. We show that ethidium monoazide (EMA) did not adequately discriminate between viable and membrane-compromised cells, whereas the propidium iodide analog PMA was excluded from viable cells irrespective of the growth and metabolic state of the cells. Besides, and yet shown for other bacteria, the amplicon size of the target sequence was critical for efficient signal reduction from dead cells. In addition, we observed that the extent of signal reduction for dead cells was dependent on the temperature, time and concentration of the dyes during staining prior to crosslink. Consistently, free protein and/or DNA present in varying amounts in the heterogenous matrix lowered the efficiency of the DNA dyes at the bacterial membrane and led to considerable variation of the residual signal from dead cells. On the basis of our findings, we propose a modified protocol, which intends to minimize assay variability and, for the first time, defines the power as well as the limits of the quantification method. This systematic approach is essential for reliable quantitative detection of viable and potentially infectious *Campylobacter* cells.

O47b - Enrichment-based Isolation of *C. jejuni* from Environmental Samples resulting in Biased Genetic Diversity

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Campylobacter jejuni is the leading cause of zoonotic bacterial gastroenteritis worldwide. The widespread occurrence of this pathogen in environmental waters, livestock and wild animal populations suggests that these sources play an important role in the epidemiology of campylobacteriosis. A comprehensive understanding of the population structure of *C. jejuni* is critical to elucidating the transmission dynamics of this important zoonotic pathogen. In an effort to explore the genetic diversity of *C. jejuni* in environmental sources, we performed 'deep' isolation experiments on water samples from several different watersheds in Canada by characterizing 100 isolates per sample. Preliminary results showed a lack of diversity among the isolates from a given sample. We hypothesized that enrichment-based isolation methods could bias the recovery towards strains with increased fitness to laboratory conditions. We have examined the effect of enrichment on the observed prevalence of different *C. jejuni* genotypes by performing competitive recovery experiments of genotypically distinct strains of *C. jejuni* from enriched and non-enriched samples and subjecting isolates recovered to high-resolution genotyping using comparative genomic fingerprinting (CGF). Our experimental results show that the distribution of genotypes recovered after laboratory enrichment is disproportionate to that of the original inoculum, with bias towards those genotypes most commonly seen in environmental sampling. These data suggest that isolation protocols utilizing enrichment for isolation of *C. jejuni* may bias the recovery towards genotypes with increased laboratory fitness. This in turn limits our current understanding of the population genetics and genetic diversity of *C. jejuni* circulating in environmental and animal reservoirs.

O48a - A Genomic Approach to the Evaluation of a Real-Time PCR Assay for Speciation of *Campylobacter jejuni* and *Campylobacter coli*.

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Routine speciation of *Campylobacter* from clinical specimens remains important for epidemiological surveillance. A real-time PCR assay developed in 2003 for speciation of *Campylobacter jejuni* and *Campylobacter coli*, based on the *mapA* and *ceuE* genes, continues to be used in clinical laboratories. The aim of the current study was to conduct a large-scale sequence-based evaluation of this assay using whole-genome sequencing (WGS) data. WGSs for 1267 *Campylobacter* isolates from the Oxfordshire Human Surveillance Project were included (http://pubmlst.org/campylobacter/projects/Oxfordshire_Human_Surveillance/). Species designations were made with multilocus sequence typing (MLST) profiles. The diversity and distribution of *mapA* and *ceuE* alleles was examined using nucleotide sequences extracted from WGS data. Additional investigations were carried out to assess the levels of similarity between *C. jejuni* and *C. coli* primer and probe sequences. *C. jejuni* and *C. coli* accounted for 90.8 % and 9.2 % of the isolates, respectively. In both species the target genes were diverse. In *C. jejuni* and *C. coli* respectively there were: 39 and 11 *mapA* alleles; and 72 and 17 *ceuE* alleles. Phylogenetic analyses indicated that, with the exception of two *mapA* alleles, all alleles were species-specific. Inspection of the nucleotide sequences revealed that primer and probe sequences were largely conserved within species. The results suggest that the assay is robust, and will be confirmed with real-time PCR. Given that the epidemiology of *Campylobacter* in Oxfordshire is representative of the UK and other developed countries, these results suggest that the assay has the potential to be applicable and reliable in many contexts.

O48b - Biofilm and its impact on the biology of *Campylobacter*

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Despite being a fastidious bacterium, best isolated under microaerophilic conditions at 42°C using specific complex media, *Campylobacter* are readily transmitted to humans from reservoir hosts. These organisms are commensal members of the gut

microbiota of animals such as wild birds, poultry, and other farm animals but in humans they can cause severe gastroenteritis. Human campylobacteriosis occurs principally via consumption of contamination food and is a worldwide problem accounting for an estimated 1,340,000 annual cases in the UK alone. The ability to survive outside of the host is a key factor in transmission and understanding how *Campylobacter* protects against environmental stresses may be helpful in reducing contamination in the food chain. We used quantitative laboratory assays to investigate differences in phenotypes - including biofilm formation, motility and aerotolerance - among isolates form multiple hosts and sources (n=273). In addition, we sequenced the genomes of these strains and analysed the evolutionary relationships among the genes involved in biofilm formation. Results were used to address two contrasting hypotheses. First, do retail meat and clinical isolates form more biofilms reflecting a strategy that allowed survival through food processing? Second, do farm and clinical strains form less biofilm - suggesting a reduction in selective pressure to promote biofilm formation, possible resulting from easier transmission between hosts in relatively densely stocked agricultural animals (compared to ancestral hosts)?

O49a - The bacteriophage carrier state of *Campylobacter jejuni*

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Bacteriophages are viruses that have the potential to control pathogenic microorganisms but understanding their complex life cycles is key to their successful exploitation. Following treatment of *C. jejuni* biofilms with bacteriophage, examination of isolated colonies revealed that phage had established a relationship with their hosts typical of a poorly understood phenomenon referred to as the carrier state life cycle (CSLC), where bacteria and bacteriophages remain associated in equilibrium. The frequency of occurrence was determined together with phenotypic changes compared to parental hosts. The effect of the CSLC on growth in different gas atmospheres and the effect on survival under adverse conditions were examined. Comparative transcriptome analyses between CSLC and parental campylobacters identified changes in gene expression associated with the presence of phage. Phage particles were found to be associated with the surface of surviving host bacteria that were non-motile and lacked flagellar structures. CSLC cultures were impaired in their ability to colonise chickens and invade intestinal epithelial cells. Significantly, CSLC *C. jejuni* exhibited increased aerotolerance under nutrient limited conditions that would confer an advantage to the survival of the *Campylobacter* in the environment. Moreover we demonstrate how CSLC host bacteria can act as an expendable vehicle for the delivery bacteriophage to new host bacteria within pre-colonised chickens. The CSLC represents an important ecological sink for *Campylobacter* bacteriophage and represents an area phage biology that has been largely neglected.

O50a - Efficacy of Selected Intervention Methods to Reduce Campylobacter Contamination on Chicken Carcasses in UK Slaughterhouses

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The effects of seven interventions were tested by either (a) treating the birds in a tunnel located pre-chill, (b) removing birds from the line pre-chill, treating them and returning to the line, or (c) treating birds ex-chill. Birds were removed from the line post-chill and tested for Campylobacter (enumeration and confirmation) on breast or back/neck skins on Days K+1 and K+7. Reductions in counts of campylobacter due to treatments with electrolysed water (up to 18 ppm chlorine), UVC (20s, 0.24 J/cm^2), and cold plasma (300s) were less than 0.3-log$_{10}$ cfu/g on breast skins. One trial with ozonated water showed no effect on Campylobacter count but the numbers were low on control birds and further work is planned. Hot water spray or dipping had an adverse effect on the appearance of the birds. Spraying with lactic acid (4%, pH=3.8) produced a 0.4-log$_{10}$ cfu/g reduction on breast skin and 0.8-log reduction on back/neck skin. Immersion of breast skins in liquid nitrogen (20s) produced a 1-log$_{10}$ cfu/g reduction. Rapid surface cooling by spraying liquid nitrogen on to birds (20s) likewise produced a 1-log$_{10}$ cfu/g reduction on back/neck skins. Trials using steam are planned. Spraying with lactic acid or liquid nitrogen show the most promise for practical application.

O51a - Polyunsaturated Fatty Acid Diets alter the immune response of chickens to *Campylobacter*

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Controlling *Campylobacter* in chickens is essential to reduce infection. *Campylobacter* is known to induce proinflammatory cytokines during infection, and to use the resulting inflammation to aid colonisation. We explored a novel broiler chicken diet supplemented with salmon oil, containing n3 Polyunsaturated Fatty Acids (PUFAs) as a method of reducing the inflammatory response to *Campylobacter* infection. Broiler chickens were stocked at commercial densities and reared on diets containing either 0.5% salmon oil or a control diet. Birds were infected with *C. jejuni* at 5, 13, 21, 29 and 36 days old. Ten birds were euthanased at 7 d intervals post-infection. The immune response in the intestinal wall of the chickens to *Campylobacter* was examined using real time PCR to a panel of cytokines and inflammatory mediators. *Campylobacter* was detected in the liver using enrichment culture and in the caecum using direct plating onto mCCDA. Both proinflammatory cytokines and inflammatory mediators were reduced in birds fed PUFAs and this correlated with a reduction in *Campylobacter* carriage. Data indicates that in order for the novel PUFA diet to be effective it needs to be used throughout rearing, irrespective of when *Campylobacter* enters the flock, and the diet is not effective in resolving pre-existing infection.

O52a - 2008–2012 fly screening ventilation inlets of broiler houses on high risk farms in Iceland to reduce flyborne transmission of Campylobacter: Impact on flock prevalence and public health.

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Introduction: Analysis of temperature-related risk factors for colonization of broiler flocks with Campylobacter in Iceland (Guerin, 2008), and findings of B.Hald in Denmark, indicated importance of fly borne transmission to flocks and potential benefit of fly screening. Due to costs associated with freezing products from flocks positive at pre-slaughter, Iceland producers were interested in fly screening highest risk farms (4–16 houses, larger flocks and high Summer flock prevalence). Methods: 35 broiler houses on 4 farms were screened in 2008, with a max of 45 houses on 7 farms in 2010. Flock results from Iceland's surveillance program were used in the analysis (pooled caeca at slaughter; direct plating on Campy-Cefex). Results/Discussion: Results for 2008, reported at CHRO 2009 showed a net 67% reduction in flock prevalence on screened broiler farms versus non-screened lower risk farms, when including historical prevalence in the analysis. For 2009–2012 we compare year by year flock prevalence on fly screened high risk broiler farms to 2002–2007 without screening. While the benefit of preventing fly borne transmission to flocks is clearly indicated by years of 0% flock prevalence on high risk farms, there is also year to year variability, suggesting inconsistency in biosecurity practices. Nevertheless, the addition of fly screening, along with emphasis on broiler house biosecurity has reduced the cost of Iceland's Freezing Policy for producers. During 2008–2012 incidence of domestically acquired campylobacteriosis was reduced on average by 90% when compared to 1999 with no controls on Campylobacter in broilers, while consumption of broiler chicken has tripled.

O53a - Combined steam and ultrasound treatment of broilers at slaughter - a promising intervention to significantly reduce numbers of campylobacters on carcasses and improve food safety

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Background: Existing steam decontamination of poultry carcasses is hampered by prolonged treatment times and adverse changes to the epidermis. In this study, a combination of steam with ultrasound (SonoSteam®) significantly reduced

Campylobacter numbers on carcasses using short treatment time and with minimal sensory changes. Methods: Full-scale equipment was installed in a Danish abattoir immediately before the inside/outside washer. *Campylobacter* were determined by direct plating on mCCD agar of skin taken from opposite sides of the breast of the same carcass before and after treatment. Sensory changes were evaluated by an authorized panel at the Danish Veterinary and Food Administration. Results: Two experiments performed on different dates, using naturally-contaminated broiler flocks showed that broilers were contaminated with *Campylobacter* levels between 2.02 and 2.67 $\log_{10}$ cfu/g (n=48). Sampling after treatment showed significant ($p<0.001$, n=12 for each experiment) *Campylobacter* reductions of 0.94 and 0.86 $\log_{10}$ cfu/g for the two experiments. Sampling after air-chilling showed significant reductions of 1.22 $\log_{10}$ cfu/g ($p<0.05$, n=12) and 0.65 $\log_{10}$ cfu/g ($p<0.01$, n=12) respectively. The effect of air-chilling without treatment showed insignificant reductions of 0.09 $\log_{10}$ compared to a mean initial level of 2.19 $\log_{10}$ cfu/g (n=12). The sensory panel concluded that chicken carcasses treated with SonoSteam were acceptable for purchase. Conclusion: The SonoSteam decontamination process can be used at slaughter to improve food safety without changing the acceptability of poultry meat, since a reduction of 1.0 $\log_{10}$ on chicken carcasses has been estimated to give a 50–90% reduction in numbers of human *Campylobacter*.

O54a - A molecular explanation for microaerophily in *Campylobacter jejuni*

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Background and Aims: Microaerophilic bacteria are adapted to life at low oxygen tensions, but the mechanisms by which their growth in air is inhibited are not well understood. We aimed to explore the molecular mechanisms responsible in *Campylobacter jejuni*. Major findings: The citric-acid cycle in campylobacters employs pyruvate and 2-oxoglutarate: acceptor oxidoreductases (Por and Oor), which contain oxygen-labile iron-sulphur centres. We have shown that these enzymes are rapidly inactivated after exposure of cells to fully aerobic conditions, with a concomitant decrease in cell viability. During microaerobic growth of strain NCTC 11168, enzyme activity is protected by the hemerythrin Cj0241. A *cj0241c* mutant exhibited an aerobic growth defect and undetectable Por and Oor activities after exposure to 21% v/v oxygen. Anaerobic recovery of enzyme activities after oxygen damage was poor, but occurred at similar rates in both wild-type and *cj0241c* mutant cells, suggesting the role of Cj0241 is to prevent Fe-S cluster damage, rather than promote repair. Another hemerythrin (Cj1224) also plays a protective role. Purified recombinant Cj0241 exhibited optical absorption and resonance Raman spectra typical of μ-oxo-bridged di-iron containing hemerythrins. Conclusions and Impact: We conclude that oxygen lability and poor repair of Por and Oor are major contributors to microaerophily in *C. jejuni*, but oxygen-binding bacteriohemerythrins help to prevent damage to these enzymes during microaerobic growth and oxygen transients. This work explains why *C. jejuni* is particularly vulnerable to molecular oxygen and highlights a new role for bacteriohemerythrins, which are widely distributed in many anaerobes and microaerophiles.

O54b - CamCon - novel approaches to control *Campylobacter* in primary poultry production

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With the aim to provide knowledge and tools to achieve production of low risk broilers (meaning broilers free of *Campylobacter* or broilers with a very low level of contamination), the CamCon project was established in 2010 under the European Community's Seventh Framework Programme. The project has partners in Norway, Denmark, UK, the Netherlands, Poland, Spain and Portugal. To understand the epidemiology of *Campylobacter* in broilers, on farm management practices in industrial chicken farms in six EU regions have been investigated (www.camcon-eu.net). These data and results from on-going longitudinal studies of flocks in UK and Spain will form the basis of a risk factor analysis of flock colonization in different climatic regions. Further, the importance of flies in transmission of *Campylobacter*, distribution of *Campylobacter* sub-types in EU, and colonization in relation to environment and welfare are being investigated. To ease sampling, a rapid method based on air-sampling has also been developed. The project also examines the effectiveness and efficacy of on-farm

interventions. Biosecurity and fly screens are being tested in the UK and Spain and phage candidates have been selected and are being tested on broiler farms in Portugal. A vaccine candidate has been developed and is being tested. The cost-effectiveness of interventions will be analysed using quantitative risk assessment models and cost calculations. Finally, to ensure dissemination of results, a web-based education programme for poultry farmers is being developed, and a certification programme will be proposed to producers and regulators. The most recent results from the project will be presented.

O55a - Transcriptome analysis of *Campylobacter* in response to bacteriophage infection

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Introduction: *Campylobacter jejuni* is the leading cause of human bacterial enteritis worldwide. Contaminated poultry meat has been found to be a major source of infection. However, previous studies show that it is possible to reduce *Campylobacter* numbers on poultry farms or during processing with the use of virulent bacteriophages. This study aims to determine the transcriptional response of *Campylobacter* to bacteriophage infection in order to give a better understanding of what occurs at the molecular level. Methods: *C. jejuni* PT14 was grown and infected with the group III bacteriophage CP30 or CPX. Samples were taken during the eclipse phase of phage growth to capture phage-infected host cells for RNAseq analysis. RNA extracted from these cells was sequenced using the Illumina HiSeq 2000 platform, which generated 50 bp paired-end reads. To identify genes regulated in response to bacteriophage infection, these reads were then mapped to the genome of *C. jejuni* PT14 using CLC Genomics Workbench. Results: Either 100 genes for CP30 or 462 genes for CPX were differentially regulated by at least 1.5 fold compared to the uninfected host. Several up-regulated genes were found to belong to the Fur and PerR regulons, therefore linking them to the regulation of iron acquisition and oxidative stress defence. Impact of research: Bacteriophage infection of bacterial cells is known to cause changes in the functioning of host cells, along with modifications during transcription and translation. This study identifies the regulatory mechanisms involved during phage infection of *Campylobacter*, and how these may impact survival or escape from phage predation.

O55b - A comparison of *Campylobacter* infection in commercial broilers under different management systems; preliminary results.

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The UK rears >850 million broiler chickens annually, with standard production systems constituting approximately 90% of production and using birds reaching slaughter weight at 35–39 days. Higher welfare (HW) systems account for the remaining 10% and typically use slower-growing breeds with lower stocking densities and are housed in houses with windows and environmental enrichment, however faster growing breeds are also grown under such systems. Previous work found that HW flocks have lower levels of *Campylobacter* and reduced incidence of welfare-associated pathologies. This project investigates whether it is broiler line that is more important in determining susceptibility to *Campylobacter* infection. We studied commercial flocks comprising four different bird lines, three fast-growing (FG1-3, slaughter weight: 35–39 days) and one slow-growing line (SG2, slaughter weight: ≥48 days). All flocks going through a single house on 15 farms (3 farms of each line plus one line (FG1) also at lower stocking density) were sampled over four crop cycles. Flocks were sampled daily, using the boot sock method, where fabric over-shoes are worn to walk through the house. Caecal counts of *Campylobacter* were also performed on batches of caeca at slaughter (first depopulation event). Preliminary data suggest that broiler line is important in determining susceptibility to *Campylobacter* infection. Significant differences were found in the levels of *Campylobacter* in the caeca, with the FG1 line having on average two orders of magnitude more *Campylobacter* than the SG1 line. Differences were also found in when the flock became *Campylobacter* positive (Average- FG1 26 days, SG1 36 days).

O56a - Characterising CHRO transcriptomes: an RNA-seq led treasure hunt

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In the past twenty years, high-throughput sequencing has revolutionised biology and allowed development of many 'omics' applications. Within the CHRO range of organisms, genome sequencing has informed us about the close evolutionary relationships between gastrointestinal colonisers like *Campylobacter* and *Helicobacter* spp, and the chemolithoautotrophic deep-sea vent colonisers like *Nitratiruptor* and *Sulfurovum*. Within genera, it is now becoming clear that there is a lot of variation in genome structure, gene sequences and gene order, which requires the mechanisms controlling gene expression to approach similar levels of flexibility. Until recently, genome-wide studies on gene expression were based on microarray technology, and this has been highly informative despite its relative low resolution and sensitivity. We are now approaching the technical limits of microarray technology, and it is now being superseded by transcriptomics based on NextGen DNA sequencing technologies (RNA-seq). The RNA-seq technology allows investigation of transcriptomes at the highest possible resolution, i.e. at the single nucleotide level. Although there are still relatively few RNA-seq based studies on CHRO organisms, they already highlight that there are many novel features hidden within CHRO genome sequences, and that there are major differences between the transcriptomes of species and even within species. Once the RNA-seq technology becomes more widely used with other members of the CHRO, we are likely to find new surprises and treasures hiding in these genomes. This presentation will contain an overview of the status of RNA-seq within the CHRO range, and include discussion of possible future directions for this exciting topic.

O56b - A mechanical hypothesis for the lag phase of Broiler colonization with *Campylobacter jejuni*

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Commercial flocks are rarely found to be *Campylobacter* positive during the first 2-weeks of production, referred to as the lag phase of colonisation. The lag phase is generally held to be the result of changes in the susceptibility of birds associated with developing immune responses and the natural gut flora, although breaches in bio-security, associated with practices such as thinning, also play an important role. However, this view of the lag phase is seemingly at odds with experimental data from challenge studies. Dose-response studies suggest that older birds are less susceptible to challenge, but constitute a larger potential for transmission. Challenge studies only assess the probability of colonisation given a fixed dose and do not account for how the risk of exposure changes over the production cycle. Mathematical models provide a means to link between experimental dose-response data and transmission at the population level. To achieve this we develop a mechanistic model of flock colonisation where transmission is mediated through an environmental reservoir that changes dynamically over the production cycle. We use this model to explore the viability of a purely mechanical hypothesis for the lag phase. We propose that the lag phase can be explained through increasing exposure, and potential for transmission, of birds as they age and perform sensitivity analyses to explore how changes in dose-response translate to the predicted duration of the lag phase. We ground-truth our model using longitudinal data and propose new observational and experimental methods to validate our hypothesis.

O57a - Comprehensive methylome analysis of the human gastric pathogen, *Helicobacter pylori*

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The genome of *Helicobacter pylori* (*Hp*) is remarkable for its large number of restriction-modification (R-M) systems, and strain-specific diversity in R-M systems has been suggested to limit transformation between unrelated strains, the major

driving force of genetic diversification in *Hp*. We have determined the comprehensive methylome of *Hp* strains 26695 and J99 at single base resolution, using single molecule, real-time (SMRT®) sequencing, which permits detection of methylation by monitoring the kinetic signal of the DNA polymerase. Three types of methylation were detected in *Hp*: N^6-methyladenine (^{m6}A), N^4-methylcytosine (^{m4}C) and 5-methylcytosine (^{m5}C). 17 and 22 methylated sequence motifs were identified for strains 26695 and J99, respectively. For most motifs, >99% of the sites occurring in the genome were detected as methylated. Our data are in overall very good agreement with previous studies of *Hp* methyltransferases (MTs) and predicted patterns of methylation. Eight novel methylation patterns were detected (26695, 3; J99, 5). Functional inactivation, correction of frame shift mutations as well as cloning and expression of candidate MTs permitted not only the identification and functional characterization of multiple yet undescribed MTs but also revealed so far unique features of Type I R-M systems. The *Hp* genome is highly methylated, and methylation patterns differ widely between strains. The methylome of the well-characterized strains 26695 and J99 will provide a valuable resource for future studies investigating the role of R-M systems in genetic diversification of *Hp* as well as the impact of DNA methylation on gene expression and host interaction.

O57b - House fly (Musca domestica) as a vector for *Campylobacter jejuni* and *Campylobacter coli* in Spanish broiler farms

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The vector potential of flies (Diptera: Brachycera) for *Campylobacter jejuni* and *Campylobacter coli* on 5 Spanish broiler farms was evaluated in a longitudinal field study from April to November in 2011 and 2012. The prevalence of *C. jejuni*- and *C. coli*-positive flies was determined in 1304 flies captured from house surroundings of the farms. Flies were macerated individually, pre-enriched in Bolton broth for 24 h at 42°C, streaked onto modified *Campylobacter* blood-free selective agar and incubated under microaerobic conditions for 48 h at 42°C. Additionally, direct PCR detection was performed from Bolton enrichment broths (2012 sampling only). Overall, 22 flies were positive by culture (*C. jejuni*, n=18; *C. coli*, n=4). *Musca domestica* (house fly) was the most frequent (89.8%) fly species captured and the only species from which *Campylobacter* was isolated. The prevalence of positive flies detected by culture was 1.7% (22/1304) with a peak in September where 31.8% (7/22) of all the positive flies were found. By PCR, overall prevalence was 10.5% (87/876), with a peak of 32.18% (28/87) of the PCR positive in August. The PCR-positive flies were mainly *M. domestica*, but also few *Ophyra* sp. (black garbage fly), *Calliphora* sp. (blow fly) and *Fannia canicularis* (lesser house fly). Most of the broiler flocks became *Campylobacter* positive around the same time or just after detecting *Campylobacter* in the sampled flies. We conclude that flies, especially *M. domestica*, near broiler houses constitute a risk for infection of broilers with *C. jejuni* or *C. coli*.

O58a - Horizontally acquired genetic elements drive genome evolution, virulence, and niche specificity in the pathogen *Campylobacter fetus.*

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Campylobacter fetus are important animal and human pathogens displaying distinct niche and host preferences. *C. fetus* subsp. *venerealis* (*Cfv*) mainly infects the bovine genital tract. *C. fetus* subsp. *fetus* (*Cff*) is less stringent and colonizes the urogenital- and intestinal-tract of animals and humans. The distinct host and tissue tropism makes *C. fetus* a particularly appropriate model to study the genetic basis of niche specificity and virulence. Horizontal gene transfer is a driving force in the evolution of pathogenic microorganisms including acquisition of virulence attributes and adaption to specific niches. The bovine isolate *Cfv* 84-112 (sap-/serotype A) was sequenced and compared to the genome of human blood isolate *Cff* 82-40 (sap-/serotype A). Although the genomes of both subspecies were found to be highly syntenic (92% sequence identity) the more niche-specific *Cfv* harbors 12% (180 kb) more genomic information than *Cff* 82-40. A circular extra-chromosomal element was identified in *Cfv* 84-112 that harbors two T4SS-related gene clusters and genes for a toxin-antitoxin system. Differences between the subspecies occur in defined hot-spots of variation encoding horizontally acquired genetic islands. Intriguingly, these genetic islands encode surface exposed structures (e.g. type IV secretion systems) or harbor various genes

involved in LPS biosynthesis. Other genes unique to *Cff* are predicted to encode CRISPR-associated proteins. An extended survey of our *C. fetus* strain collection indicated that the identified LPS-biosynthesis genes are linked to certain pathotypes. Moreover, they were shown to confer serum and acid resistance indicating their involvement in host and niche preference of *C. fetus* subspecies.

O58b - The Role of Slaughter Practices in the Transfer of *Campylobacter* Contamination between Batches

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The slaughter of *Campylobacter* positive birds may contribute to the spread of *Campylobacter* towards broiler carcasses and the slaughterhouse environment. A highly contaminated production line could be the source for *Campylobacter* transfer from one batch to another. Therefore, this study aimed to assess the role of slaughter practices in the *Campylobacter* cross-contamination between batches in two slaughterhouses. During every visit, 3 successive batches were investigated. Carcasses (n=2) were collected at four locations on the slaughter line after 1, 10 and 20 min from the start of the slaughter of each followed batch. Additionally, six caeca contents per batch were collected to determine the initial *Campylobacter* colonization level. *Campylobacter* was quantified by direct plating on CampyFood Agar® (bioMérieux SA, France) plates. *Campylobacter* was not detected on carcasses (LOD=10 cfu/g) when only *Campylobacter* negative birds were slaughtered. However, when *Campylobacter* positive batches entered the slaughter line, an immediate increase in the carcass contamination (> 4 log cfu/g) was observed. When all slaughtered batches were positive, the concentration of *Campylobacter* recovered from carcasses remained at a high level (>3 log cfu/g). When a *Campylobacter* negative batch followed a positive one, a rapid decrease in *Campylobacter* carcass contamination was observed over time in both slaughterhouses. If only *Campylobacter* negative batches are slaughtered, non-contaminated carcasses are produced. The slaughter of positive batches results in contaminated carcasses across the slaughter line. *Campylobacter* is only transmitted in relatively low numbers from a positive to a subsequent negative batch, and the transmission level decreases rapidly over time.

O59a - A genome-wide association study of *Campylobacter* survival in the poultry processing chain

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Pathogenic bacteria typically pass through several intermediate habitats as they are conveyed from the reservoir niche to human infection. The ability to survive these stages is a fundamental property associated with the emergence of zoonotic pathogens and tracing the genetic changes associated with this may help in developing infection control plans. A promising approach, pioneered in human genetics, is genome-wide association mapping where DNA sequence variation across the genome is related to particular phenotypes. This approach has been challenging to apply to bacteria because of their strong population structure resulting from clonal reproduction, but here we present a new method and apply it to investigate genetic variation in *Campylobacter* as it passes through the food chain. Contaminated poultry meat is a major source of human infection and the strains infecting humans are considered to be a subset of those found in chickens on the farm. To better understand the genes and alleles associated with survival at different stages, we sequenced more than 500 *Campylobacter* genomes from chickens on the farm, abattoirs, retail poultry meat and human disease. The genomes were divided into overlapping 30bp words, allowing simultaneous analysis of homologous and non-homologous sequence variation and words that were significantly overrepresented in particular stages were highlighted. This provided a list of candidate genes and functions that could be important in the passage of *Campylobacter* from farm to human disease, some of which were tested and confirmed *in vitro*.

O59b - An integrated model to estimate the source of *Campylobacter* infection in broiler houses

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This study determines the relative importance of the sources (e.g. wild birds and farm animals) of *Campylobacter* in broilers using a modelling approach. A Monte Carlo (MC) transmission model was developed and parameterised using datasets on both dose response in broilers and survival of *Campylobacter* in the environment. The survival of *Campylobacter* was determined for a representative selection of sequence types (STs) and environmental matrices (e.g. animal faeces, soil and water). It showed either log-linear or log-linear with shoulder decay processes. The environmental infection pressure at the broiler house depended upon the heterogeneous nature of *Campylobacter* excreted by animals and birds, the different rates of ST decay, the distance between the broiler house and location of animals as well as the type and size of the animal reservoir. Exponential dose response models in broilers were determined using published data and by animal feeding studies. The infectious doses (ID_{50}) were as low as 180 cfu, but varied depending on the ST and the source of the isolates. The estimated percentage of broiler houses becoming infected decreased as the distance between an animal field and the broiler house increased. Cattle and sheep were equally important colonisers, but wild birds were also important due to their close proximity to the broiler houses. Further work will consider the effect of removal of farm animals, controlling birds and reducing the re-seeding of the environment by the broiler chickens themselves.

O60a - The pathogenomics of *Campylobacter jejuni* in New Zealand

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New Zealand has one of the highest notification rates of human campylobacteriosis in the world with, at one point, ~25–30% of these cases due to a pre-eminent multilocus sequence type (MLST) *C. jejuni* strain called ST-474 that is rare outside NZ. Previous comparative genomic analysis has shown that two isolates of ST-474 showed sequence differences in ~5% of their genes. We performed a comparative study of 62 *Campylobacter* genomes from a variety of species (*C. jejuni, C. coli*, as well as 3 putative new *Campylobacter* species) isolated from different NZ sources to investigate the genome-wide population structure and evolutionary forces at play in a relatively isolated island ecosystem. DNA from *Campylobacter* spp. isolates were sequenced using Illumina technology and draft genomes generated. Techniques such as Markov clustering of gene predictions and pairwise genome comparisons were used on various subsets of the genomes in order to visualise the core genome of these bacteria, and to assess the relative contributions of recombination and mutation. We have also used a new synthetic MLST scheme called rMLST (ribosomal MLST) to look at genomic relationships at both the allelic and nucleotide level. Sequence comparisons using NeighborNets show that the 52 rMLST genes are a good proxy for much larger core genome sets, depending on the species under investigation. The visualisation method Circos has also been used to show where areas of recombination tend to cluster in the genome, and that recombination is responsible for generating more diversity than mutation.

O60b - A large scale survey describing the relationship between broilers and human campylobacteriosis

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Aim: Campylobacteriosis was the most commonly reported zoonosis in the EU in 2012. This study aims to trace *C. jejuni* strains along the poultry meat production chain from farms to retail stores and to determine their implication in campylobacteriosis in France. Method: The MLST method was used to investigate the circulation of *C. jejuni* genotypes in the French broiler food chain up to the consumers. Isolates from farms (176) and slaughterhouses (302) were collected during a survey sampling representative of the French broiler production in 2008. Retail meat isolates (175) were collected in 10 French departments representing the most significant consumption patterns over the year 2009. Human isolates (143) were collected in 2009 from patients living in the same areas in order to study the overlap between broiler and human

isolates. Major findings: Among the 796 isolates collected, 595 have been typed and a great diversity has been observed with 157 different STs of which 112 (493 isolates) have been assigned into 30 ST-complex. Among the isolates from broiler meat production, three larger complexes predominated: ST-21 (18.58%), ST-45 (15%) and ST-464 (6.9%). Among the human isolates, the most common was also ST-21 (36.4%), followed by ST-206 (9.8%) and ST-48 (7.7%). Impact of research: This is the first large scale survey in France involving representative number of isolates from poultry and humans. The results confirm that poultry meat production remain a substantial source of human infections in France. ST-21 complex is the predominant complex in both broiler and human isolates.

O61 - What has the Campylobacter jejuni genome done for us?

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It is 12 years since the first Campylobacter jejuni full genome sequence was published and it perhaps timely to ask what the genome sequence has done (and not done) for us. The immediate benefit of having the NCTC11168 genome sequence was the identification of genes and genetic loci previously unknown. The most striking examples included the presence of a capsule and a novel N-linked general glycosylation system, which allowed for the first time the production of recombinant glycoproteins with wide ranging implications in the design of glycoconjugate vaccines. The absence of enterotoxins, effector molecules and type III and IV secretions system was equally striking. The sequencing of further genomes and the application of DNA microarrays revealed a highly diverse species with multiple mechanisms for the generation of genetic diversity. This diversity was evident in strains that had essentially been amplified and perpetuated by the livestock industry in the industrialized world. More recently, the sequencing of strains from more diverse sources and geographic locations has revealed further diversity, typified by the presence of type VI secretion systems particularly from developing world isolates. More recently, RNA seq approaches have revealed small RNA sequences and CRISPR-systems in several strains and computational/systems biology approaches have helped to determine gene function, particularly in polysaccharide pathways. This lecture will summarize the salient features of C. jejuni omics technology and will reflect on the challenge of determining gene function and relating this to the commensal lifestyle of C. jejuni in avians and in causing disease in humans.

O62 - Genome variation in Helicobacter pylori

Sebastian Suerbaum

O63 - From MLST to genomics: the gene-by-gene approach to population annotation for Campylobacter.

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Genomics provides great opportunities for the development of the organismal approach to the study of *Campylopbacter*. The structured gene-by-gene cataloguing of population variation, or 'population annotation', using online resources such as http://PubMLST.org/Campylobacter will enable the synthesis of concepts, ideas, and techniques from different disciplines. Indeed, with this approach, the very diversity of *Campylobacter* populations, so long a hindrance to our understanding, provides tremendous scientific opportunities. We now know, for example, that certain *Campylobacter* genotypes are associated with particular hosts, with different wild bird species, for example, being associated with particular genotypes. An intriguing exception to this is the existence of certain 'multihost' genotypes which are found in chicken and ruminant sources, which perhaps represent organisms that have evolved since the advent of farming or intensive farming methods. It is even possible that the introgression of *C. jejuni* genes into *C. coli* clade one is an evolutionary consequence of expansion into a novel agricultural niche. Whatever the ultimate causes of the genetic and phenotypic diversity, its existence provides the prospect of identifying the genomic determinants for particular phenotypic properties using gene association studies. The diversity of the candidate genes identified can be explored in populations in terms of sequence variation and distribution and their biological and immunological roles probed in functional investigations. However, there are challenges as well as opportunities ahead as the maintenance and expansion of online resources such as the PubMLST databases, which are 'public goods', will need community support and contribution if they are to survive and flourish.

O64a - Investigation of the alterations in the phase variable genes of *Campylobacter jejuni* 11168 during colonisation of chickens

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Campylobacter jejuni is the most common bacterial cause of infectious gastroenteritis. The main disease source is contaminated poultry, and *C. jejuni* asymptomatically colonises the gastrointestinal tract of chickens to very high levels. One aspects of *C. jejuni* biology that has received limited attention is phase variation (PV) of surface structures. The genome of *C. jejuni* strain NCTC11168 contains 27 loci that are subject to PV due to alterations in polyG repeat tracts. PV of these loci causes modification of surface structures - capsule, lipo-oligosaccharide and flagella - often targeted by host adaptive immunity. The aim of this project is to investigate whether PV plays a role in immune avoidance and host colonization. Chickens were inoculated with strain NCTC11168. Output populations and serum samples were collected after two and six weeks of persistence in the chickens. A method for measuring changes in all 27 polyG tracts was developed and will be utilised for examining the input and output populations from the *in vivo* experiment. These findings will indicate the contributions of PV to host colonisation whilst comparison of expression states with immune targets will determine whether PV contributes to immune escape.

O64b - The novel *Campylobacter jejuni* effector protein CiaD induces mitogen-activated protein kinase signaling pathways required for host IL-8 secretion

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Acute infection with *Campylobacter jejuni* causes severe diarrhea containing blood and leukocytes. Key to the mechanism of pathogenesis is the delivery of the *Campylobacter* invasion antigens (Cia) to the host cells through the flagellar Type Three Secretion System (T3SS). Our lab has recently identified a novel Cia protein, termed CiaD, that possesses a putative mitogen-activated protein kinase docking motif. We hypothesized that CiaD is delivered to the host cytosol where it is involved in manipulating host cell signaling events. We found that CiaD is delivered to the host cell in a flagellar dependent manner. Similarly, we evaluated the role of CiaD during the induction and secretion of the proinflammatory chemokine interleukin-8 (IL-8). We show that the *ciaD* mutant induces significantly less secretion of IL-8 from the host cell when compared to the *C. jejuni* wild-type strain. Similarly, we evaluated the role of CiaD in modulating host cell signaling, and found that the *ciaD* mutant strain was deficient in activation of the host cell signaling components p38 and Erk 1/2 (MAPK signaling molecules). Finally we show that CiaD is necessary for the development of acute disease *in vivo*. Based on these data, we concluded that CiaD is delivered to the host cell cytosol through the flagellar T3SS, and is required for the induction of host IL-8 secretion and the development of acute disease.

O65a - Phenotype variation of *Campylobacter jejuni* sequence types associated with wild birds and ruminants

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Campylobacter jejuni is a thermophilic species that grows well at 42°C, a temperature associated with the avian body, and does not actively grow below 30°C, excluding *C. jejuni* subsp. *doylei*. We examined the phenotypic pattern of six *Campylobacter* sequence types (ST) that are associated with different hosts, at two temperatures, 22°C and 42°C. ST42 has been isolated worldwide and is associated with ruminants and human disease. The ST2381 clonal complex has not yet been reported outside of New Zealand, and is associated with two species of indigenous New Zealand birds and with surface water. The genome of each isolate was sequenced. Using a phenotypic microarray (Omnilog system) we examined the carbon utilisation pattern of isolates from ST42 and the ST2381 clonal complex. The genomes were used to identify the genetic components of phenotypic differences. In general, isolates utilised fewer carbon sources at 22°C and these are a subset of those utilised

at 42°C by the same isolate. ST42 isolates tended to utilise a wider range of carbon sources at 42°C than did the ST2381 clonal complex isolates. These results suggest that the carbon utilisation pattern may reflect adaptation to different hosts. It also suggests that *C. jejuni* may reduce or change its metabolic activity at lower temperatures i.e. when being transmitted from host to host. These metabolic changes could represent useful targets for interventions aimed at inactivating C. jejuni in food stored at lower temperatures.

O65b - Novel mechanisms of IL-8 induction by CagL of the *Helicobacter pylori* type IV secretion apparatus

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The *cag* pathogenicity island (PAI)-encoded type IV secretion system (T4SS) of *Helicobacter pylori* triggers a massive inflammatory response during infection of the gastric epithelia by a mechanism not fully understood. Previous studies showed that the *H. pylori* protein CagA and the cell wall component peptidoglycan, when delivered into the host cell by the T4SS, activated transcriptional factor NF-kB leading to induction of the potent proinflammatory chemokine interleukin-8 (IL-8). However, deletion of *cagA* and knockdown of the intracellular peptidoglycan-sensing nucleotide-binding oligomerisation domain 1 (NOD1) receptor fails to completely reduce IL-8. It has long been speculated that the T4SS apparatus itself might contribute to IL-8 induction but direct proof was lacking. Here we reveal that a non-effector T4SS surface component, CagL, contributes directly and predominantly to the induction of IL-8. Inhibition of a_5b_1 integrin or mutation of the arginine-glycine-aspartate (RGD) motif in CagL significantly attenuated IL-8 induction. CagL-directed induction of IL-8 involved activation of integrin a_5b_1, Src kinase, Ras GTPase, the mitogen-activated protein kinase (MAPK) pathway and NF-kB. Remarkably, exogenous CagL dramatically increased the ability of the *H. pylori* mutant P12D*cagL*, but not the P12D*cag*PAI mutant, in activating NF-kB and IL-8 secretion, suggesting that CagL functions synergistically with the rest of the T4SS in triggering proinflammatory responses. This occurred independently of CagA translocation and NOD1-dependent signaling. These findings reveal a previously uncharacterised innate immune mechanism whereby the *H. pylori* T4SS apparatus is recognised by the host adhesion receptor integrin b_1 to mount a potent proinflammatory response.

O66a - Genomic diversity among five Campylobacter jejuni chicken isolates representing the ST-677 clonal complex

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To study the genetic diversity and microevolution of *Campylobacter jejuni* we used whole genome sequencing by Illumina and PacBio RS to describe chicken isolates simultaneously collected from the inside (chicken fecal sample) and outside of chicken houses (water puddle and/or sock sample) at two farms in Finland. All three isolates from one farm represented ST-677 and two isolates from another farm represented ST-794. The ST-677 clonal complex (CC), in which both STs belong to, has been common among Finnish chicken and patient isolates. Previously only one draft genome of a ST-677 isolate (LMG 9872) from a meningitis case from Sweden has been sequenced. Illumina sequencing resulted in 34–36 contigs for each of the five farm isolates. The draft genomes were annotated using the RAST annotation system. Genes and genetic subsystems shared with reference strains will be discussed as well as unique features for the ST-677 CC. The most evident differences between the two different STs and geographically separated ST-677 isolates were observed in phage-related genomic regions. Breseq will be used to analyze the microevolution (SNPs, phase variation) of *C. jejuni* isolates among the different farms, and between the ST-677 isolates from our study compared to the isolate of the same ST, LMG 9872, isolated 25 years earlier. All the isolates were found to represent the LOS class O. Frequencies of the length variations of the homopolymeric tracts in orf23o and orf25o will be discussed. These and further results from both phenotypic and comparative genomics analysis will be presented.

O66b - Pathogen comparative genomics and host transcriptomics to investigate the pathogenic potential of Campylobacter concisus

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Introduction: Campylobacter concisus has received increasing attention over the last decade and has been described as an emergent pathogen of the intestinal tract. C. concisus has been associated with two types of intestinal diseases, gastroenteritis and inflammatory bowel diseases, major causes of morbidity and mortality. Aim: To employ genomics to determine virulence genes within C. concisus strains and host transcriptomics to elucidate the host response to C. concisus infection. Methods and Results: Illumina HiSeq 2000 sequencing coupled with Velvet assembly and RAST annotation was employed to sequence, assemble and annotate seven C. concisus genomes (three Crohn's disease, three gastroenteritis and one healthy control). Comparative genomic analyses of eight genomes (including the reference strain) identified virulence genes associated with the pathogenic potential of the strains. Moreover, these analyses revealed amino acid changes within host-related proteins that may be associated with adherence, invasion and motility. Illumina MiSeq sequencing chemistry was employed to determine the host epithelial response to C. concisus infection. Reads were mapped to all available features of the human genome and raw counts were evaluated using Benjamini Hochberg adjusted t-tests. This identified ~500 human genes that had significant changes in expression (>2-fold, <0.5-fold) upon infection with C. concisus. Moreover, non-coding RNA and somatic mutations putatively associated with infection have been identified. Conclusions: This study provides novel information on the genetic make-up of C. concisus strains and the global epithelial response to infection by this bacterium, adding significant support to the view that C. concisus is an emerging intestinal pathogen.

O67a - *H. pylori* strain dominance via transformation

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Introduction: Helicobacter pylori can be divided into distinct phylogeographic populations, and in Latin America, H. pylori isolates of the population hpEurope are increasingly dominant at the expense of the Amerindian hspAmerind. We hypothesized that genomes from hspAmerind that evolved from a small founder population have lost cognate recognition sites (CRS) for restriction and/or methylation enzymes promoting hpEurope's DNA invasion. Methods: CRS and active methylases will determine direction and frequency of gene flow during transformation and thus, we determined: i) the observed and expected frequencies of CRS in 110 multilocus DNA sequences and 7 whole genomes from different H. pylori populations; ii) number of active methylases (by resistance to in vitro digestion by 16 restriction enzymes of genomic DNA) in hpEurope and hspAmerind strains; iii) the direction of DNA uptake in co-culture experiments of hspAmerind and hpEurope strains. Results: We found that most CRS were consistently underrepresented between whole genomes and multilocus DNA sequences. The frequency of CRS or number of active methylases (average 8.6 + 2.6) did not differ between bacterial sub-populations, but hspAmerind and hpEurope strains showed different restriction profiles, with 15 recognition sites accounting for the differences. Amerindians strains exhibited higher transformation rates than European strains, and were more susceptible to be subverted by larger DNA hpEurope-fragments than vice versa. Impact: The geographical variation in the pattern of CRS provides evidence for ancestral differences in RMS representation and function, and the transformation findings support the hypothesis of Europeanization of the Amerindian strains in Latin America via DNA recombination.

O67b - Induction of exopolymeric matrix in response to a host-specific signal in Campylobacter jejuni

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A biofilm is defined as a surface-associated community of bacteria surrounded by a hydrated extracellular polymeric matrix (EPM). Although several studies have reported biofilm formation by Campylobacter jejuni, the EPM has not been characterised. We show that exposure to physiological concentrations of a host intestinal protein (HIP) leads to a change in the profile of exported proteins and to secretion of an extracellular polysaccharide comprising an alpha-1,6-glucan that remains loosely associated with the cell surface. Cj0511, encoding a secreted protease, is essential for this response and we demonstrate degradation of HIP by recombinant Cj0511. Induction of the EPM in response to HIP leads to an increase in biofilm formation in vitro and to increased virulence in a Galleria mellonella infection model. Pre-exposure of C. jejuni to HIP also results in a significant increase in the colonisation of chickens and promotes tolerance of environmental stress. These data are important for understanding the process of colonisation by C. jejuni and has implications for the design of Campylobacter control strategies in food animal production.

O68a - Chromosome painting in silico in a bacterial species reveals fine population structure

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Identifying population structure forms an important basis for genetic and evolutionary studies. Most current methods to identify population structure have limitations in analyzing haplotypes and recombination across the genome. Recently, a method of chromosome painting *in silico* has been developed to overcome these shortcomings and has been applied to multiple human genome sequences. This method detects the genome-wide transfer of DNA sequence chunks through homologous recombination. Here, we apply it to the frequently recombining bacterial species *Helicobacter pylori*, which has infected *Homo sapiens* since their birth in Africa and shows wide phylogeographic divergence. Multiple complete genome sequences were analyzed including sequences from Okinawa, Japan, that we recently sequenced. The newer method revealed a finer population structure than revealed by a previous method that examines only MLST housekeeping genes or a phylogenetic network analysis of the core genome. Novel subgroups were found in Europe, Amerind and East Asia groups. Examination of genetic flux showed some singleton strains to be hybrids of subgroups and revealed evident signs of population admixture in Africa, Europe, and parts of Asia. We expect this approach to further our understanding of intraspecific bacterial evolution by revealing population structure at a finer scale (Molecular Biology and Evolution, 2013).

O68b - Modulation of the Helicobacter pylori Type IV Secretion System Function in Response to Adaptive Immune Pressure

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Introduction: Virulent *Helicobacter pylori* expresses a Type IV secretion system (T4SS) encoded by the *cag* pathogenicity island (PAI), which is required to translocate CagA and induce IL-8. *cagY* is an essential component of the PAI that has extensive repetitive sequence motifs that can recombine and eliminate or restore function of the T4SS. Here we characterize the immune mechanism that selects non-functional *cagY* alleles. Results: *H. pylori* strains isolated from C57BL/6 but not RAG-/- mice showed a marked decrease in the induction of IL-8 and translocation of CagA, which was accompanied by

recombination in *cagY*. Similar to RAG-/- mice, T cell knockout mice failed to select for low IL-8 inducing strains or change in *cagY*, whereas output strains from B cell knockout mice resembled those from C57BL/6 mice. Adoptive transfer experiments confirmed that CD4+ T cells alone are sufficient to select *cagY* variants that eliminate T4SS function. We next challenged IFNg-/- and IL-10-/- mice to provide a reduced and enhanced inflammatory environment, respectively. Strains recovered from IL-10-/- mice lost the ability to induce IL-8 and changed *cagY* faster and more completely than strains from C57BL/6 mice. In contrast, strains recovered from IFNg-/- remained unchanged, indicating that selection for *cagY* variants is IFNg mediated. Conclusions: We propose that immune pressure mediated through IFNg selects for variation in CagY, yielding a pool of strains with altered inflammatory potential. We speculate that this variation permits *H. pylori* to adapt to changing pro inflammatory conditions within the gastric environment in order to maximize persistent infection.

O69a - Comparative genome analysis identified horizontally acquired genes important for virulence and niche adaptation in *Campylobacter fetus* and *Campylobacter curvus*.

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Campylobacter fetus subsp. *venerealis* (*Cfv*) is highly niche adapted, infecting the genital tract of cattle. *C. fetus* subsp. *fetus* (*Cff*) is less stringent in host and tissue preference, colonizing the urogenital- and intestinal-tract of animals and humans. *Cff* is an emerging human pathogen causing severe systemic diseases and death. *C. curvus* (*Cc*) is considered an oral *Campylobacter* species involved in the development in periodontitis. It can also cause severe systemic disease. Although both species are distinct in tissue and host preferences they share the ability to survive within the gastrointestinal tract and to cause invasive infection in humans. To study intra- and interspecies differences, whole genome comparisons were performed. Comparative analysis of *Cfv* strain 84-112 and *Cff* strain 82-40 revealed horizontally acquired genes (*mat1*, *glf* and *wcbK*) involved in lipopolysaccharide (LPS) biosynthesis. PCR screens showed that these genes are distinctively distributed within *Cfv*, *Cff* sero-/sap-type A and B strains. Interestingly, *wcbK* (encoding a putative GDP-mannose 4,6-dehydratase) was identified only in *Cff* sero-/sap-type B strains. Mutational inactivation of *wcbk* disrupted LPS production, impaired acid resistance of the strain and rendered the bacteria resistant to human serum. Sequencing and comparison of two *Cc* isolates derived from human systemic diseases (intra-abdominal and liver-abscess) also identified putative LPS-biosynthesis genes. Given that LPS and the S-layer are important virulence factor and that S-layer structure is determined by the bacterial LPS, the identified LPS-biosynthesis genes in both species are interesting candidates to study virulence and niche adaptation in Campylobacter.

O69b - RNA-seq of Campylobacter jejuni using a novel *in vivo* bile model

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Introduction: Recent advances in the use of high throughput deep sequencing of RNA (RNA-seq) have revolutionized the study of gene expression and identification of small non-coding RNAs in pathogenic microorganisms. Unlike *Helicobacter pylori*, there is minimal published data on small RNA transcription in *Campylobacter jejuni*. In addition, most published reports of RNA-seq in bacteria have involved description of only a single growth condition or of differential gene expression using *in vitro* models. Methods: Here, we have developed a novel *in vivo* model using the sheep gallbladder to allow for exposure of *C. jejuni* to a bile-rich host environment and collection of high quality total RNA following adaptation. Strand specific RNA-seq was then performed on the Illumina Hi-seq to analyze total RNA expression from both the *in vivo* and *in vitro* environments. Results: The data obtained from the sequencing of total RNA isolated from *C. jejuni* clearly demonstrates the existence of previously unknown non-coding RNA transcripts as well as a surprisingly complex yet ordered antisense transcriptional landscape of unknown function. In addition, assessment of differential gene expression using QuasiSeq has yielded over 400 genes differentially expressed in the *in vivo* bile environment. Impact of research: This research provides valuable insight into the mechanisms that may be utilized by *C. jejuni* to induce disease and develop a carrier state within the inhospitable bile environment. To share this data with the rest of the *Campylobacter* research community, a Gbrowse web interface is being developed for enhanced visualization of the RNA-seq data.

O70a - Genome sequence of Campylobacter phage CP21

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Campylobacter is one of the most important foodborne pathogens worldwide. Infections are mainly caused by the consumption of undercooked meats, especially poultry. To control Campylobacter within the food chain, application of virulent bacteriophages is a promising tool. Though, a thorough understanding of the biology and genetics of the phages is mandatory. One major issue concerns the genomes of the phages which have to be stable and free from critical genes (e.g. toxin genes). In this study we present the genome sequence (182,833 bp) of Campylobacter group II phage CP21 and show that group II phage genomes are very similar in sequence but may be different in terms of modular organization. The CP21 genome is composed of four large modules separated by long repetitive sequences. Similar modules and repeats occur in the group II phages CP220 and CPt10 but the modules are divergently arranged in CP21. We propose the repeat regions of group II phages to be substrates for recombination causing extensive genomic rearrangements.

O70b - A study of formation, carbohydrate composition and mechanical forces exhibited by *Helicobacter pylori* biofilm

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Helicobacter pylori has been postulated to survive in extra-gastric environments within biofilm structures. This study investigates the development of biofilm formation and carbohydrate composition of *H. pylori* NCTC11637 biofilms over time. In addition, the mechanical forces exerted on AGS cells upon *H. pylori* infection were examined. *In vitro* biofilm formation by *H. pylori* was measured using a modified crystal violet staining assay while biofilm development was examined using scanning electron (SEM) and confocal laser scanning microscopy (CLSM). Size exclusion chromatography (SEC), gas-chromatography mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) were used to elucidate the biofilm components. The forces exerted by *H. pylori*-infected AGS cells were analyzed using an array of force microsensors. SEM reveals that initial biofilm formation is strain dependent. *bio*Image-L analysis of Live/Dead staining viewed under CLSM indicates *H. pylori* remains viable for weeks within the biofilm structures. NMR indicates the presence of proteomannans. Monosaccharide analysis of extracted extracellular polysaccharides of Day 14 biofilms indicated that mannose is the major sugar (80%). GC-MS of sugar linkages revealed that biofilm contained 1,3-mannosyl,1,4-mannosyl and terminal mannosyl linkages. AGS cells infected with 7 day-old planktonic *H. pylori* exhibited twice the forces that of bacteria within biofilm. Our studies show that mannose is a major sugar component of NCTC11637 biofilms. Studies are on-going to better understand the role of proteomannans in biofilm formation. The force microsensor study demonstrates the potential of this technique to study the infectivity of *H. pylori* and this approach can be used to study pathogen-host mechanical interactions.

Posters

Pathogenesis

P1. Resistance to six antimicrobial agents in *Helicobacter pylori* clinical isolates in Spain.

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The aim of this study was to determine the resistance of Helicobacter pylori clinical isolates to six antimicrobial agents. Material and Methods: gastric biopsies from 429 patient were studied. H. pylori was cultured following standard microbiological procedures and susceptibility testing was performed by E-test. EUCAST breakpoints were used for definition of susceptibility and resistance. Results: Clarithromycin resistance was found in 52% of strains and 1% were intermediate. Tetracyclin and amoxicillin resistance was found in 1% and 13%, respectively without differences according to sex or age. Metronidazole resistance was 35% without differences according to sex, resistance was higher in adults (44%) than en children (33%) ($p<0.05$). Levofloxacin resistance was 93%, being higher in adults (25.9%) than in children (3,4%) ($p<0.01$). Rifampicin resistance was 28% without differences according to sex or age. Conclusion: Clarithromycin and metronidazole resistance was high in our H. pylori clinical isolates. Resistance to metronidazole and levofloxacin was higher in strains from adults than in that from children.

P2. New 3D-infection models based on tissue-engineering to study pathogenesis of *Helicobacter pylori* and *Campylobacter jejuni*

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About 50% of the world's population is infected with *Helicobacter pylori*, the causative agent of gastritis, ulcers, and gastric cancer. The related Epsilonproteobacterium, *Campylobacter jejuni*, is currently the most common cause of bacterial gastroenteritis. Like for many other human pathogens, no optimal animal model that comprehensively mimics the human disease is available for these two prevalent pathogens. Therefore, infection studies have mainly employed animal or *in vitro* cell-culture models, which are limited in their ability to reflect the *in vivo* situation in the human host and to model the complexity of an intact three-dimensional (3D) tissue. Here we aim to develop new human 3D *in-vitro* infection models based on tissue engineering to study host-pathogen interactions and virulence mechanisms of *Helicobacter* and *Campylobacter*. Tissue engineering has already been successfully applied to create replacement structures for reconstructive surgery. Applied *in vitro*, these complex 3D tissue-cultures can mimic the microenvironment of human tissues. First, as a proof-of-principle, we will use our previously developed small-intestine model to establish *Campylobacter* infections. Furthermore, we aim to develop a novel stomach tissue-model to study *H. pylori* infections. Analysis of deletion mutants or different strains in these new infection models can then be used to identify and characterize novel virulence genes and phenotypic differences among isolates. The new model systems will also be useful for infection studies with other pathogens and the development of new therapeutic and diagnostic tools.

P3. *Helicobacter pylori vac*A polymorphism in dyspeptic Ghanaian patients

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Aim: This study assesses the clinical significance of *vac*A strains in *Helicobacter pylori* isolates from Ghanaian patients with gastro-duodenal disease. Methods: This study utilised a cross-sectional design to sample consecutive patients with gastro-duodenal disease at the Korle-Bu Teaching Hospital between 2010 and 2012. Antral biopsies were taken for rapid-urease (CLO) testing and additional samples stored in DNA-gard solution. DNA was extracted from tissue samples using a commercial kit and stored at -20oC until PCR analysis. *Vac*A specific gene primers were used to amplify the signal and middle regions of the *vac*A gene. Major findings: The study sampled 242 patients however; 93 patient samples have so far had *vac*A PCR characterization. The *Helicobacter pylori* prevalence was 74.2% and the endoscopic diagnoses encountered were gastritis (42%), gastric ulcer (29%), duodenal ulcer (26%) and gastric cancer (9%). *Vac*A s1 was detected in 62% of patients, *vac*A m1 in 53% and *vac*A m2 in 7%. The *vac*A s1m1 genotype was identified in 38% of participants and *vac*A s1m2 in 5%. The *vac*A s1m1 genotype was demonstrated in all four diseases [gastric cancer (50%), duodenal ulcer (45.8%), gastritis (35.9%) and gastric ulcer (11.1 %)]. Main conclusion: *Helicobacter pylori* gastro-duodenal disease is common in Ghana. The highly virulent *vac*A s1m1 strain was very prevalent across most sub-groups in comparison with *Vac*A s1m2. Impact of Research: This is the first such determination on *Helicobacter pylori vac*A polymorphism in Ghana. It risk stratifies patients with *Helicobacter pylori*-gastro-duodenal disease regarding virulence and oncogenicity in an endemic area.

P4. Acid Adaptive Mechanisms of *Campylobacter Jejuni* in The Gastrointestinal Tract

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Introduction: *Campylobacter jejuni* is a major cause of acute bacterial diarrhea in humans worldwide. The mechanism of *C. jejuni* acid-survival is poorly understood. We aim to investigate how *C. jejuni* survives acidic conditions and to characterize the role of acid-stress in the pathogenesis of *C. jejuni*. Methods: The acid stimulon of *C. jejuni* was characterized by microarray approaches. Disk inhibition assays were used to evaluate the sensitivity of acid-stressed *C. jejuni* to H_2O_2. KatA expression was determined using western-blot analysis and qRT-PCR. Acid-stressed and unstressed *C. jejuni* were tested for adherence and invasion to Caco-2 cells. Finally, the role of acid-stress in *C. jejuni* pathogenesis was determined using the *G. mellonella* infectious model. Results: Exposure of *C. jejuni* to acid stress enhanced its ability to survive oxidative stress. Western-blot analysis and qRT-PCR demonstrated that acid stress increased *katA* expression. Moreover, exposure of *C. jejuni* to acidic conditions resulted in up-regulation of genes involved in acid survival and pathogenesis. In agreement with this observation, acid-stressed *C. jejuni* exhibited increased ability to adhere and invade Caco-2 cells. In addition, the pathogenesis of *C. jejuni* in *G. mellonella* was significantly enhanced upon exposure to acidic conditions. Conclusions: Prior exposure of *C. jejuni* to acid-stress enhances pathogenesis and cross-protects Campylobacter against other stresses.

P5. *Campylobacter jejuni* Lipooligosaccharide Sialylation, Phosphorylation and Amide/Ester Linkage Modifications Fine-Tune Human Toll-Like Receptor 4 Activation

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Aims: To determine if LOS structural variation seen amongst *C. jejuni* strains reflects their ecological source and their ability to modulate TLR4 function. Methods: MALDI-TOF Mass Spectrometry (MS) was performed to characterize structural variation in both the oligosacchride (OS) and lipid A (LA) components of 15 *C. jejuni* isolates. Cytokine induction from THP-1 cells and monocytes was correlated with LOS structural variation in each strain. Major Findings: OS sialylation,

increasing abundance of LA D-glucosamine (GlcN) *versus* 2,3-diamino-2,3-dideoxy-D-glucose (GlcN3N) and phosphorylation status all correlated with TLR4 activation as measured in THP-1 cells and primary monocytes. Importantly, LOS-induced inflammatory responses were similar to those elicited by live bacteria highlighting the prominent contribution of the LOS component in driving host immunity. OS sialylation status but not LA structure showed significant association with strains clustering with livestock sources. Conclusions: Our study highlights how variations in three structural components of C. jejuni LOS alter TLR4 activation and consequent monocyte activation.

P6. Antibiotic Susceptibility Patterns of *Campylobacter coli* and *Campylobacter jejuni* in South African free range and commercial chicken

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Introduction: Many countries have banned the use of antibiotic growth promoters but South Africa continues to use them intensively. Globally, there have been increased reports of *Campylobacter* strains of broiler origin being resistant to antibiotics within the fluoroquinolone and macrolide classes of antibiotics. The aim of this study was to determine the susceptibility of *Campylobacter coli* and *C. jejuni* isolated from South African free range and commercial chicken to antibiotics commonly administered for human therapeutic purposes. Methods: Antibiotic susceptibility testing was carried out for nalidixic acid, tetracycline, erythromycin and ciprofloxacin according to the disk diffusion method and results were interpreted according to the guidelines of the NHLS (SA). Results: Of free range origin, *C. coli* strains (n = 33) were 18, 39, 61 and 85% resistant to the above-mentioned antibiotics respectively, while *C. jejuni* strains (n = 56) were resistant by 4, 11, 50 and 64% respectively. *C. coli* isolates (n = 4) of commercial chicken origin were 100, 50, 100 and 25% resistant to the antibiotics respectively while *C. jejuni* strains (n = 29) showed 100, 86, 97 and 79% resistance to the antibiotics respectively. Impact of research: The generally higher incidence of antibiotic resistance observed in commercially bred chickens may indicate the unregulated use of antibiotics on South African poultry farms. The high incidence of antibiotic resistance in *Campylobacter* strains of chicken origin is reflected in the clinical *Campylobacter* strains isolated from Cape Town patients with campylobacteriosis.

P7. Functional analysis of the *Campylobacter jejuni N*-linked glycan

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Campylobacter jejuni, a gastrointestinal pathogen in humans and a commensal of poultry, was the first bacterium demonstrated to possess a general N-linked protein glycosylation pathway. Although >60 proteins become *N*-glycosylated little is known about the role of the *N*-glycan. We demonstrate that the *N*-glycan on CmeA, a component of the *C. jejuni* multidrug efflux pump, protects this protein from degradation. Unglycosylated, mono- and double-glycosylated CmeA expressed in *E. coli* lacking the major periplasmic protease, DegP, were stable whereas over-expression of DegP led to degradation of the unglycosylated and N273 mono-glycosylated CmeA. In contrast, double-and N123 mono-glycosylated CmeA were not degraded indicating that *N*-glycosylation at N123 is important for protection. Interestingly, CmeA from *Campylobacter fetus fetus* becomes glycosylated at two different sites (N275, N249) and is a substrate for DegP and DegQ. Here, protection is conferred through *N*-glycosylation at site N275. In *C. jejuni,* loss of CmeA glycosylation led to decreased survival in the presence of antibiotics and bile salts, and to reduced levels of colonization in chickens fed with antibiotics providing evidence that *N*-glycosylation of CmeA contributes to the stability of the CmeAB-TolC efflux pump. Using Molecular Dynamics simulations we demonstrate that the *N*-glycan shields large portions of CmeA; but the *N*-glycan does not change the overall protein conformation. However, gel filtration experiments using purified CmeA showed that CmeA *N*-glycosylation promotes dimerization of the protein. Our data demonstrate that *N*-glycosylation protects CmeA from proteolytic digestion and promotes protein dimerization and protein complex stability probably due to glycan-protein interactions.

P8. *Helicobacter pylori* mediated disruption of mitochondrial dynamics reveals a new mechanism for mitochondrial-dependent cell death during infection of gastric epithelial cells

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Aims. Recent studies revealed that *Helicobacter pylori* (*Hp*) infection of gastric epithelial cells disrupts the physiologically important process of mitochondrial dynamics by a VacA-dependent mechanism. While the consequences of disrupting filamentous mitochondrial networks during *Hp* infection are not fully understood, excess mitochondrial fission precedes and is important for induction of cell death. An increase in cell death within the gastric mucosa may both promote persistent *Hp* infection while at the same time increasing the risk for gastric cancer. The aim of the current study was to identify the mechanism by which *Hp* mediated disruption of mitochondrial dynamics promotes cell death. Methods. Fluorescence imaging and biochemical approaches were used to identify and characterize the mechanism by which VacA-mediated disruption of mitochondrial dynamics results in a loss of mitochondrial outer membrane integrity, thereby committing *Hp*-infected cells to undergoing programmed cell death. Major Findings. Our studies revealed that VacA-mediated disruption of mitochondrial dynamics is transduced by the cytosolic cellular stress sensor protein Bid, which was activated in response to excess mitochondrial fragmentation. Once activated, Bid mobilized the cellular machinery required to breech the integrity of the mitochondrial outer membrane as a requisite step preceding cell death. Surprisingly, Bid activation did not occur by known mechanisms, but instead occured as a direct result of VacA dependent fragmentation of filamentous mitochondrial networks. Main Conclusion and Impact of the Research. Our results indicate a new mechanism by which pathogen-mediated disruption of mitochondrial dynamics directly engages the cellular apoptotic machinery.

P9. Properties of the truncated form of *Helicobacter pylori* Dsb protein - oxidase HP0231.

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Aims. The mechanism of disulfide bond formation in microorganisms is extremely diverse and the well-characterized *E. coli* Dsb system can no longer be treated as a model for other bacteria. Our recent work led to the characterization of the first dimeric oxidoreductase (HP0231). It is intriguing that HP0231 acts as periplasmic oxidase, as EcDsbA, despite its structural resemblance to EcDsbG. However, the characteristic motifs of HP0231 are identical to that of EcDsbA (CPHC/VcP) but different from that of EcDsbG (CPYC/TcP). To assess relations between HP0231 structure and its oxidizing activity we tested whether truncated form of HP0231 containing only catalytic domain and lacking dimerization domain and α linker acts as periplasmic oxidase in *H. pylori* and *E. coli* cells. Methods. All genetic manipulations were performed using standard molecular biology procedures. A shuttle vector expressing HP0231 and its mutated form were introduced into *H. pylori* N6 *hp0231::cat* or in *E. coli dsbA::kan/dsbAdsbB::kan1,2* cells and complementation tests were performed. Major findings. Truncated form of HP0231 restores alkaline phosphatase activity and motility in *E. coli dsbA*$^-$ cells but not in *dsbAB*$^-$ cells. The protein also does not restore motility and cadmium resistance in *H. pylori hp0231::cat* cells. Main conclusion. Truncated form of HP0231 complements lack of DsbA in *E. coli* cells in a DsbB dependent manner. However, potentially it is not able to cooperate with HpDsbI (homolog of EcDsbB).

P10. Characterization of CagL, an outer protein of the *Helicobacter pylori* Cag-type IV secretion system, by chromosomal site-directed mutagenesis

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The genome of virulent *Helicobacter pylori* (*Hp*) strains contains the *cag* pathogenicity island (*cag*PAI) that plays a crucial role in the outcome of *Hp* infection. A subset of this genetically highly variable region encodes the Cag-type IV secretion system (T4SS). During infection, *Hp* employs the Cag-T4SS to translocate effectors (e.g. CagA, peptidoglycan) into epithelial cells, which can lead to malignant host cell transformation. Additionally, *cag*PAI positive *Hp* strains induce the secretion of pro-inflammatory chemokines such as interleukin-8 (IL-8). We focused our research interest on the HP0539/*cagL* gene

product, a T4SS tip protein involved in IL-8 induction in host cells. Based on 3D structural modeling and analysis of global interstrain variation, we identified several domains which could be involved in host cell interaction or type IV secretion system function. In order to characterize CagL protein function in detail, we constructed isogenic chromosomal non-marked mutants containing site-directed deletions or amino acid substitutions in various regions. These mutants were tested for CagL expression, localization, secretion and integrin interaction. Furthermore, the influence of single CagL mutations on CagI expression, a protein that directly interacts with CagL, was investigated. In order to reveal functional deficits in the respective mutants, we performed cell infection experiments and CagA translocation assays. To gain further insight into the role of CagL in pathogen-host interaction, we cloned V5-tagged HP0539 and neighbouring upstream genes into a shuttle vector for complementation studies. In this context, differences in pathogen-host interaction of the generated mutants and functional characteristics of CagL will be discussed further.

P11. Epithelial invasion and pathogenicity of *Campylobacter fetus* subsp. *venerealis* involves a putative invasin

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Introduction: *Campylobacter fetus* subsp. *venerealis* (*Cfv*) causes the statutory disease bovine venereal campylobacteriosis and epidemic abortion in cattle. Mutational inactivation of the type IV secretion component *virD4* in virulent *Cfv* isolates was reported to attenuate invasion and cell-killing in human cell lines. This study analyzes the role of distinct virulence factors on pathological changes in intestinal epithelia. Methods: Isogenic knock-out mutants of *Cfv* strain 84-112 were generated by insertion of a kanamycin-cassette in either *invA*, *cdtB*, or *virD4*. Human colonic epithelial monolayers (HT-29/B6, Caco-2) were apically inoculated with the different *Cfv* strains. Transepithelial electrical resistance (TER) and tracer fluxes were measured in Ussing chamber experiments. Invasion was determined by gentamicin protection assay. Bacterial translocation across the cell monolayer was counted. Apoptosis induction was detected by TUNEL-staining. Tight junction proteins were analyzed by Western blotting and their subcellular distribution by confocal laser-scanning microscopy. Results: An MOI of 200 of the *Cfv* wild type isolate decreased TER in epithelial cell monolayers 6h after infection. Translocation of bacteria was 4% of the apical bacterial number in Ussing chambers supplied with microaerobic atmosphere. 24h post infection the wild type strain induced epithelial apoptosis, epithelial lesions and cell detachment. The *Cfv_ΔinvA* strain was attenuated in decreasing epithelial resistance at 24h and invasion at 2h p.i. when compared to the wild type strain. Impact of research: The putative invasin of *Cfv* seems to play a critical role in epithelial damage and bacterial translocation across epithelial barriers. Supported by grants from the Deutsche-Forschungsgemeinschaft(DFG_Schu_559/11) & the Austrian-Science-Fund (FWF_P20479-B05).

P12. The role of autophagy in the pathogenesis of *Campylobacter concisus*

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Background and Aims: *Campylobacter concisus* is an emerging gastrointestinal pathogen with studies showing associations with both Gastroenteritis and Inflammatory Bowel Diseases. Given the importance of autophagy for the elimination of intracellular bacteria and the link between autophagy and Crohn's disease, we investigated the importance of autophagy in the pathogenesis of the invasive strain *C. concisus* UNSWCD. Methods: In order to assess intracellular *C. concisus* levels within infected Caco-2 cells, we used both standard and modified Gentamicin protection assays. The effect of autophagy induction and inhibition, on intracellular survival was also investigated. To assess the interaction between *C. concisus* UNSWCD and autophagosomes, confocal microscopy, scanning electron microscopy, and transmission electron microscopy (TEM) were employed. Moreover, autophagy gene expression levels were measured using PCR arrays. Major Findings: Autophagy inhibition and induction resulted in significant changes in the intracellular levels of *C. concisus* UNSWCD as compared with controls (no inhibitor or inducer). Investigation of *C.concisus* UNSWCD survival showed that it was present

intracellularly within host cells over an extended period of time. However, under autophagy inhibition, intracellular survival of *C. concisus* UNSWCD decreased dramatically. Confocal microscopy showed co-localisation between the bacterium and autophagosomes, which was also observed via TEM. Gene expression results showed that following infection, key genes involved in the autophagy process were significantly regulated. Main Conclusion and Impact of Research: In conclusion, this study provides a possible mechanism whereby *C. concisus* UNSWCD might manipulate the autophagy system to benefit its intracellular survival. These findings further support the pathogenic potential of *C. concisus*.

P13. *N*-linked protein glycosylation in *Wolinella succinogenes*

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Prokaryotic protein glycosylation is an increasingly reported phenomenon. A bacterial *N*-linked protein glycosylation (pgl) system was first reported in *Campylobacter jejuni* and similar pathways have been identified in related organisms. One such related species is *Wolinella succinogenes*, the only species of the *Wolinella* genus and a member of the Epsilon subdivision of the Proteobacteria, along with *Campylobacter* and *Helicobacter* spp. Although a number of features are common to both the *C. jejuni* and *W. succinogenes pgl* loci, the latter also contains a number of additional uncharacterised genes. Three approaches to characterise *N*-linked protein glycosylation in *W. succinogenes* were undertaken. In the first approach, *W. succinogenes* membrane preparations were shown to glycosylate an *N*-linked glycosylation sequon-containing peptide and mass spectrometry revealed the structure of the *W. succinogenes*-derived hexasaccharide glycan. In the second approach, the role of *pgl* genes in *N*-glycosylation was investigated using a previously described fosmid containing the complete *W. succinogenes pgl* locus that functions in *Escherichia coli*. Using insertional mutagenesis we have elucidated the role of pgl gene products involved in sugar biosynthesis and glycan transfer. In a third approach, genetic complementation of a *C. jejuni pglB* mutant with the *W. succinogenes pglB* resulted in *N*-linked glycosylation of a distinct restricted set of *C. jejuni* proteins. These data indicated different functions of the *W. succinogenes* and *C. jejuni pglB* genes and combined they provide an insight into bacterial *N*-linked glycosylation systems beyond the *C. jejuni* based prototype.

P14. Dual infection dynamics of *Campylobacter jejuni* strains in a commercial line of chickens in an experimental setting.

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Although many commercial broiler flocks are dominated by a single *Campylobacter jejuni* genotype, in some flocks multiple genotypes can be isolated. However, little is known about the infection dynamics of different genotypes within individual birds and whether different genotypes colonise different sites within the GI tract. We examined how two *C. jejuni* strains disseminate and colonise birds in an experimental Ross 308 broiler flock (n=20) and determined whether different strains colonise different sites within the GI tract. At three weeks of age, two birds were infected with 10^7cells of *C. jejuni*-M1(ST137) and two with 10^7 *C. jejuni*-13126(ST21). The remaining 16 birds were unchallenged. Cloacal swabs were taken at 2, 5, 8, 12 and 15 days post-infection (dpi) to determine semi-quantitatively the levels of each strain. At 18dpi, all birds were killed and in half we quantified the levels of each strain at nine sites within the GI tract and liver. In the remaining birds, the levels were quantified in only the caeca and liver. At 2dpi, 8/19 birds were shedding *C. jejuni*-M1 whereas none were shedding *C. jejuni*-13126. By 8dpi all birds were shedding both strains. At 18dpi, *C. jejuni*-13126 was found throughout the GI tract, whereas *C. jejuni*-M1 was found mainly in the caeca and colon. The livers of 7/19 birds were positive with *C. jejuni*-13126 whilst none were positive with *C. jejuni*-M1. These results suggest *C. jejuni*-13126 may be a more invasive strain and able to infect the liver, as well as the whole of the GI tract.

P15. Chemotaxis transducer in *Campylobacter jejuni* with a role in sensing essential nutrients

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Chemotaxis mediated motility enables *Campylobacter jejuni* to navigate through various environmental gradients and allow for its establishment in diverse niches. *C. jejuni* genome has 11 putative Methyl Accepting Chemotaxis proteins (MCP) and some of these MCPs have been identified for sensing aspartic acid, formic acid, and for energy taxis. However, the ligands for many other MCPs have not been described. Besides, the nutrient specificity of MCPs and their role in *C. jejuni* pathobiology remain to be elucidated. Here we describe the characterization of a MCP2 with its unique properties in sensing essential nutrients. Novel to our findings, the Δ*mcp-2* mutant showed a decreased chemotaxis towards inorganic phosphate (Pi) and iron (Fe^{2+}). Promoter fusion assays demonstrated that P_{mcp-2} was induced in the presence of 2mM Pi and 40μM iron. The promoter activity was abolished in a *C. jejuni* mutant for the Ferric uptake regulator protein (Fur). Additionally, real time PCR revealed an upregulation in phosphate uptake genes in the Δ*mcp-2* mutant. Primer extension assays revealed a promoter approximately 52 bp upstream to the *mcp-2* gene, and reverse transcriptase PCR demonstrated that *mcp-2* is co-transcribed with another gene involved in phosphate metabolism. The Δ*mcp-2* displayed a growth defect under nutrient downshift and iron restricted conditions. The mutant was also defective in chicken colonization but was increased in intracellular survival in INT 407 cells. Our findings reveal novel ligands for MCP-2 in *C. jejuni* which can interact with underlying pathways of iron and phosphate regulation, thus enhancing our understanding of *C. jejuni* biology.

P16. Investigation of the interaction node of *Helicobacter pylori* flagellar biogenesis protein HP0958.

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Aims: Motility is an important feature of *Helicobacter pylori* infection. HP0958 is a flagellar biosynthesis protein which is essential for motility. HP0958 stabilises RpoN, the sigma factor controlling expression of Class II flagellar genes. HP0958 also interacts with the *flaA* mRNA transcript, encoding the major flagellin, FlaA. The crystal structure of HP0958 revealed two domains: an N-terminal α-helical coiled-coil and a C-terminal Zn-finger domain. This structural data has provided information that has informed our design of mutations to test interactions with protein and mRNA. Methods: Site-directed mutagenesis of HP0958 was performed to identify potential residues involved in interactions with the flaA mRNA transcript and RpoN. The HP0958/*flaA* mRNA interaction was investigated by electrophoretic mobility shift assay (EMSAs). The HP0958/RpoN interaction was investigated by yeast two-hybrid assay followed by enzyme assay. A panel of *hp0958* mutants were re-introduced into an *hp0958-null* mutant strain of P79; effects on expression of Class II and Class III flagellar genes were monitored by western blot. Major findings and conclusions: A panel of HP0958 mutants were generated based on their potential role in protein-protein/protein-RNA interactions. A number of candidates have been identified as involved in the interaction of HP0958 with RpoN and the *flaA* mRNA transcript. Impact of research: Construction of the bacterial flagellum is a complex, hierarchical process involving over 40 proteins; regulation in *Helicobacter* differs from well-studied model organisms, *E. coli* and *S. enterica* serovar Typhimurium. Understanding the mechanism by which HP0958 contributes to this complex process will improve our understanding of these differences.

P17. iTRAQ analysis of Campylobacter jejuni prophage effects on protein expression associated with the virulence and biology of the organism

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Aims: Temperate bacteriophages are present within the genome of Campylobacter jejuni. One of these prophages, CJIE1, was associated with increased adherence and invasion using INT-407 cells in culture. We assessed the quantitative changes in the proteome of very closely related isolates using iTRAQ labelling and nano-LC MS/MS in the presence and absence of

CJIE1 prophage under different growth conditions. Methods: Four very closely related C. jejuni isolates were chosen for analysis; three carried the CJIE1 prophage, while the fourth lacked it. Trypsin digested proteins were labelled with 4-plex iTRAQ kit, fractionated using high-pH reversed-phase liquid chromatography (RPLC) and analyzed by low-pH nano-RPLC/MS/MS. Results were analyzed with Mascot and Scaffold software. Major Findings: Only 47 of ~1300 proteins were differentially regulated by more than log2 $\geq$ 0.6 in the isolates carrying CJIE1 versus the single isolate that did not. Of these, 11 were consistently detected after growth in three different media: Oxoid Mueller-Hinton agar containing 10% sheep blood, Mueller-Hinton agar, and Mueller-Hinton agar containing 0.1% sodium deoxycholate. These proteins included putative virulence proteins and other proteins that may have key regulatory or metabolic roles. Prophage-encoded proteins were detected only in isolates carrying CJIE1. Further experiments demonstrated a difference in the expression of iron acquisition proteins when growth on media with and without blood as well as the existence of a bile response comprising a majority of proteins. Impact of research: Quantitative proteomics has been shown to be useful for elucidating mechanisms underlying bacterial virulence and for characterizing bile and iron responses.

P18. Pathogenic potential of *Arcobacter* strains isolated from edible bivalve molluscs

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Bivalve shellfish have been found to contain a high prevalence of the food-borne pathogen *Arcobacter* spp. However, the pathogenic potential of the recovered isolates is unknown. Therefore, our main goal was to assess the presence of virulence genes and the antimicrobial susceptibility of *Arcobacter* strains recovered from edible bivalve molluscs. A total of 96 shellfish samples (including clams, razor clams, surf clams, mussels, scallops and oysters) retailed between 2010 and 2012 in Chile, were analyzed by culture. The obtained colonies were identified by multiplex PCR and PCR-RFLP, nine putative virulence genes (*cadF*, *ciaB*, *cjl349*, *irgA*, *hecA*, *hecB*, *mviN*, *pldA* and *tlyA*) were assessed by PCR and the antimicrobial resistance was tested by the dilution agar method. The global *Arcobacter* incidence was 35.4%, with the highest occurrence in surf clams (75.0%) followed by razor clams (65.0%), mussels (38.8%), clams (23.8%) and oysters (15.0%). The most prevalent species was *A. butzleri* (55.4%) followed by *A. cryaerophilus* (27.0%), *A. skirrowii* (16.2%) and *A. defluvii* (1.4%). A high resistance was found to nalidixic acid, while intermediate resistance was observed to ciprofloxacin, levofloxacin and ampicillin. No resistance was found to erythromycin and gentamicin. In relation with virulence, the predominant detected genes were *mviN*, *ciaB*, *tlyA*, *pldA* y *cadF*, while none of the strains possessed the *irgA* gene. These results are similar to those reported previously in clinical strains, which demonstrate the pathogenic potential of arcobacters isolated from shellfish and suggest that this type of food could be one of their transmission routes.

P19. Cj1411c encodes for a membrane associated cytochrome P450, from *Campylobacter jejuni* 81-176, directly involved in virulence

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Campylobacter jejuni is the most commonly recognized cause of bacterial gastroenteritis in humans and especially in children. Cytochromes P450 are widespread in nature and have remarkably diverse functions at both the biochemical and physiological levels. The genome of *C. jejuni* 81-176 encodes a single cytochrome P450 (Cj1411c) that has no close homologues. Cj1411c is unusual in its location within the capsule polysaccharide biosynthesis cluster suggesting a possible association within this surface structure. Use of an anti-Cj1411c antibody has enabled us to confirm its outer surface localization. We have expressed the Cj1411c protein in *E. coli* and shown it to be a lipophillic P450, containing one equivalent of Cys-ligated heme. Cj1411c activity is controlled via the N-glycosylation pathway and is posttranslationally regulated by tyrosine phosphorylation. A Cj1411c deletion mutant had significantly reduced ability to infect human cells compared to the wild type strain. This study describes the first membrane associated bacterial cytochrome P450 directly involved in virulence.

P20. Biosynthesis of modified heptoses from the capsule of *Campylobacter jejuni*.

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The capsule of *Campylobacter jejuni* is important for colonization and virulence in various infection models. In most strains, the capsule comprises a modified heptose whose role and biosynthesis are unknown. We show that biosynthesis of 6-deoxy-D-*altro*-heptose involves sequentially the GDP-D-glycero-*manno*-heptose 4, 6-dehydratase WcbK, and Cjj1430 and Cjj1427 but does not directly involve the capsular C4 reductase WcaG. While a predicted C3/C5 epimerase, Cjj1430 only serves as a C3 epimerase and the putative C3/C5 epimerase / C4 reductase Cjj1427 only serves as a reductase along this pathway. Using the WcbK reaction product as a surrogate substrate, we characterized three enzymes involved in L-*gluco*-heptose synthesis: Cj1427, Cj1428 and Cj1430. We show that despite high levels of similarity with *altro*-heptose synthesis enzymes, these enzymes have pathway-specific catalytic activities and substrate specificities. Cj1430 has both C3 and C5 epimerisation activities and Cj1428 is only a C4 reductase but is specific for the C3/C5 epimer generated by Cj1430. Cjj1430, Cj1430, Cjj1427 and Cj1428 are the only members of the C3/C5 epimerases / C4 reductases family that only exhibit one of their multiple potential activities and show activity on heptose. However, apart from Cjj1430, none of them is highly specific for heptose. Finally, we show that Cj1427 is not necessary for L-*gluco*-heptose synthesis and does not affect its production while its homologue WcaG regulates the production of D-*altro*-heptose. These studies expand our fundamental understanding of heptose modification, provide new glycobiology tools for biomedical applications, and provide a foundation for the structure function analysis of these enzymes.

P21. Differential expression in a ciprofloxacin-resistant *Campylobacter jejuni* strain under short-term and long-term ciprofloxacin pressure

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Aims: *Campylobacter jejuni* ATCC 33560 is the reference strain for antimicrobial susceptibility testing. Ciprofloxacin, a fluoroquinolone, is one of the antibiotics of choice in treating *Campylobacter* infections, but resistance to ciprofloxacin is increasing and seems to persist without antimicrobial pressure. We sought to find underlying bacterial factors, other than the well-known *gyrA* mutations, involved in ciprofloxacin resistance. Methods: A ciprofloxacin resistant mutant of *C. jejuni* ATCC 33560 was created by growing this strain under ciprofloxacin exposure (1 μg/ml). Microarray analysis, utilizing probes based on the sequences of *C. jejuni* NCTC 11168, 81-176 and RM1221, was performed on the wild-type (WT) strain, the ciprofloxacin mutant grown under normal conditions (RM) and the ciprofloxacin mutant exposed to 32 μg/ml ciprofloxacin during growth (RP). To confirm microarray results of targets of interest, qRT-PCR is being performed with primers developed based on the ATCC 33560 genome sequence. Results: Differential expression was found for genes involved in iron metabolism, which generally were downregulated in the RP strain compared to the RM strain. Genes involved in efflux systems were commonly upregulated in the RP strain. qRT-PCR experiments are underway to confirm the results of the microarray. Impact and conclusion: Ciprofloxacin resistance is an increasing problem in western countries. Isolates resistant to ciprofloxacin persist for a long time, even without antibiotic pressure. By studying the gene expression of ciprofloxacin-resistant *C. jejuni* and the effect of antibiotic exposure, the underlying bacterial factors involved in this resistance may be explained and potential targets for future antimicrobial treatment may be found.

P22. *Lactobacillus* inhibits adherence of *Helicobacter pylori* to host cells by interfering with host cellcalcium signalling

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Aims: The bacterial communities we are harboring play an important role in controlling the colonization of pathogens and preventing infections. In this study, we investigated how lactobacilli influence adherence of *H. pylori* to host cells. Methods: Adhesion and cell signaling assays, as well as RNA interference and inhibition assays were used. Major Findings: We found that certain lactobacilli reduce adherence of *H. pylori* by affecting host cell signaling pathways. We demonstrate that inhibitory lactobacilli counteract pathogen-induced activation of Src, subsequent Ca2+ release from the endoplasmic reticulum and

upregulation of the calcium responsive gene *EGR1*, in a Toll-like receptor 2 dependent manner. Main conclusion: The results of this study reveal that certain lactobacilli, but not all, reduce pathogen adherence by counteracting host cell signaling induced by the pathogens. Impact of Research: These data argue that the composition of the *Lactobacillus* flora may play an important role in blocking host cell signaling pathways required for colonization of pathogenic bacteria.

P23. Effects of stress-adaptation on Campylobacter jejuni

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Campylobacter jejuni, the major cause of human gastroenteritis, is a fragile bacterium requiring special conditions in the laboratory for its growth. In nature, however, this organism is able to survive in very diverse and hostile environments and produce disease in humans. The mechanisms that the organism has evolved for its survival in stressful conditions are not fully understood. An adaptive tolerance response (ATR) has been found to help this bacterium in surviving stresses. In order to determine the effect of acid-adaptation and exposure to secondary stresses on virulence, the expression of virulence genes as well as adhesion and invasion on INT 407 cells were studied on various C. jejuni strains after exposure to an acid pH of 5.5 for the acid-adaptation and then re-challenged with secondary stresses such as acid (pH 4.5), salt (3% NaCl) and starvation (phosphate buffered saline pH 7.2). Acid-adapted cells were found to have higher survivability, adhesion and invasion and up-regulation of some virulence genes, but these were found to be dependent on factors such as the type of isolate, time of acid-adaptation and type of secondary stress. These results indicate that C. jejuni surviving stress may be more resistant to further stress such as passage through the human gastrointestinal tract and that stress may be a significant factor in inducing some virulence genes.

P24. The Twist in the Gut: The Influence of Chicken Mucin on *Campylobacter jejuni* Virulence

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Background: *Campylobacter jejuni* naturally colonises chickens in high numbers but no disease occurs in these avian hosts. Transmission of the organism to humans occurs via contaminated poultry where penetration of the mucosal barrier results in invasion of the underlying intestinal epithelium and gastroenteritis ensues. Differences in the composition of mucins between these species may play a role in determining disease outcome. This study aimed to examine differences in binding of *C. jejuni* to chicken versus human mucin. The effect of purified chicken mucin on *C. jejuni* virulence gene expression was also investigated. Methods: Binding of *C. jejuni* strain 81-176 to purified chicken mucins and to mucin purified from a human colonic cell line was analysed using a novel microarray platform and surface plasmon resonance analysis. *C. jejuni* was grown in the presence and absence of purified chicken mucins and reverse transcriptase PCR was carried out to assess mRNA expression of a number of virulence genes. Results: *C. jejuni* bound to chicken and human mucin immobilised on both the mucin microarray and Biacore chip and exhibited a distinct tropism for chicken mucin compared to mucin purified from the colonic cell line. Co-culture of *C. jejuni* with purified chicken mucin resulted in altered expression of key genes implicated in pathogenicity. Conclusion: The strong affinity of *C. jejuni* for chicken mucin together with the finding that chicken mucin can modulate the expression of genes involved in virulence may explain why *C. jejuni* behaves as a commensal in these animals.

P25. The Role of TFF1 in the Interaction of *Helicobacter pylori* with Epithelial Cells

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Introduction: *Helicobacter pylori* exhibits tropism for mucus secreting cells which produce the mucin MUC5AC and TFF1, a member of the trefoil factor family of proteins. *H. pylori* is the only bacteria shown to date to interact with a trefoil factor. We aimed to assess the effect of TFF1 on the interaction of *H. pylori* with epithelial cells. Methods: HT29-MTX cells, colonic adenocarcinoma cells that secrete TFF1, and AGS-AC1 cells, a clone of the gastric AGS cells which can be induced to express TFF1, were infected with *H. pylori* and numbers of adherent organisms were calculated. The amount of IL-8 produced was quantified and RT-PCR was used to assess the effect of infection on gene expression. Results: Expression of TFF1 by cells resulted in higher numbers of wild type bacteria interacting with the cells compared with cells that do not produce TFF1. Infection of cells with a *H. pylori* LPS mutant, unable to bind TFF1 did not result in enhanced infection of cells expressing TFF1. The addition of copper to the cell culture medium in order to promote dimerization of TFF1 enhanced infection even further. However, the level of IL-8 produced by cells upon infection was not influenced by expression of TFF1. Infection of AGS cells induced enhanced TFF1 mRNA expression. Impact of Research: The results of this study suggest that *H. pylori* exhibits a tropism not just for cells that produce MUC5AC but also for cells expressing TFF1. Modulation of TFF1 expression by *H. pylori* may aid acute infection.

P26. A prospective follow-up study on transmission of *Campylobacter* from poultry to abattoir workers

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Poultry meat is an important source of campylobacteriosis and genotypes of human isolates have been shown to overlap considerably with those of poultry origin. However, it is not clear whether all chicken *Campylobacter* isolates can be transmitted to humans and cause disease. We studied the transmission of *Campylobacter* from chicken flocks to abattoir workers during the summer months June to September, the peak season for human infection. In this prospective follow-up study, 28 initially stool culture negative abattoir workers were sampled once a month. Data from the participants were obtained by questionnaires. Follow-up samples revealed positive *Campylobacter* cultures from 7 abattoir workers, 5 in September. No symptoms of infection were reported by any of the *Campylobacter* positive participants although 4 of them were employed within a year from the beginning of the study and had not reported any previously documented *Campylobacter* infection. Five of the isolates were typed to *C. jejuni* and one to *C. lari*. Multilocus sequence typing revealed that the *C. jejuni* isolates belonged to clonal complexes previously found in patients with symptomatic infections. Four of the human isolates had *Sma*I restriction patterns identical to that of chicken isolates from flocks slaughtered within one month of human infection. This study provides evidence for zoonotic transmission of *Campylobacter* from chicken to humans at poultry abattoirs. The results suggest that asymptomatic *Campylobacter* infection might occur even in individuals without earlier detected campylobacteriosis. Transmission from chicken to human was accompanied with a genetic change in the lipooligosaccharide locus of one isolate.

P27. Gut microbiota composition is associated with susceptibility to *Campylobacter* infection

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The role of the intestinal microbiota in colonization resistance to enteropathogens in humans is not understood. In this prospective follow-up study we addressed the gut microbiota composition in relation to *Campylobacter* infection by 16S amplicon sequencing of stool samples from 17 abattoir workers. Participants were followed between June and September, with an additional stool sample collected in February. Seven individuals became infected with *Campylobacter* during the study period but had no symptoms and 10 uninfected participants were selected as controls. The microbiota was dominated

by *Bacteroides*, *Ruminococcaceae* and *Prevotella,* known as main contributors of the three human enterotypes. *Campylobacter* positive individuals had a significantly higher abundance of *Bacteroides* compared to the non-infected control group, whereas the latter had an over representation of *Clostridiales* and *Lachnospiraceae*. Intriguingly, the *Campylobacter* infected group had significantly higher abundance of intestinal *E. coli* compared to the controls. This is in line with earlier findings in mouse models, that a high abundance of *E. coli* decreases colonization resistance to Gram negative pathogens such as *Salmonella enterica* and *Campylobacter jejuni*. Intra-individual comparisons of the gut microbiota composition yielded small differences over time in uninfected controls. However, significant differences were detected between samples separated by more than 5 months among *Campylobacter* positive individuals. This suggests that *Campylobacter* infection influences the gut microbiota composition. To the best of our knowledge, this is the first study showing that the composition of the human gut microbiota can affect colonization resistance to *Campylobacter* and that microbiota composition is affected by this infection.

P28. Intracellular trafficking of *C. jejuni* in *A. polyphaga*

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Campylobacter jejuni is able to enter, survive and multiply within the free living amoeba *Acanthamoeba polyphaga*, but the molecular mechanisms behind these events are still unclear. We have studied the uptake and intracellular trafficking of viable and heat killed bacterial cells of the *C. jejuni* strain 81-176 in *A. polyphaga*. We found that viable bacteria associated with a substantially higher proportion of *Acanthamoeba* trophozoites than heat killed bacteria. Furthermore, the kinetics of internalization, the total number of internalized bacteria as well as the intracellular localization of internalized *C. jejuni* were dramatically influenced by bacterial viability. Viable bacteria were internalized at a high rate already after 1h of co-incubation and were observed in small vacuoles tightly surrounding the bacteria. In contrast, internalization of heat killed *C. jejuni* was low at early time points and did not peak until 96h. These cells were gathered in large spacious vacuoles that were part of the degradative pathway as determined by the uptake of fluorescently labeled dextran. The amount of heat killed bacteria internalized by *A. polyphaga* did never reach the maximal amount of internalized viable bacteria. These results show that the massive bacterial invasion of *A. polyphaga* observed in co-cultures with *C. jejuni* requires bacterial viability, indicating that this process is bacterially induced. Furthermore, viable *C. jejuni* could escape degradation in *A. polyphaga* by avoiding localization to lysosomal vacuoles.

P29. *Campylobacter jejuni* outer membrane vesicles possess proteolytic activity and enhance bacterial adhesion to and invasion of intestinal epithelial cells

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Campylobacter jejuni produces outer membrane vesicles (OMVs) that play a role in pathogenesis. Previously we have shown that 151 proteins are associated with *C. jejuni* 11168H OMVs (including 16 *N*-linked glycoproteins and two proteases HtrA and Cj0511) and that *C. jejuni* OMVs are able to induce a host innate immune response from T84 intestinal epithelial cells (IECs). Further studies have demonstrated that *C. jejuni* 11168H OMVs contain biologically active proteases that degraded specific substrates (casein and gelatin). Quantitative protease profiling in the presence of phenylmethanesulfonyl fluoride (PMSF) significantly inhibited *C. jejuni* OMV protease activity. PMSF is a serine protease inhibitor and Cj0511 is annotated as a serine protease. The role of serine proteases in *C. jejuni* pathogenesis is still unclear. Pre-incubation of Caco-2 IECs with *C. jejuni* OMVs before co-culture with live *C. jejuni* results in increased numbers of both interacting and intracellular bacteria, compared to cells not pre-incubated with OMVs. It is possible that the proteolytic activity of *C. jejuni* OMVs may play a role in damaging the surface of IECs to promote bacterial adhesion and invasion. These data indicate that *C. jejuni* OMVs promote *C. jejuni* interactions with host cells.

P30. *In vivo* colonisation of *Campylobacter jejuni* 11168 wild type and *corA* mutant in the chicken infection model

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A number of human infections acquired through farm environment sources such as poultry, pork and its products, and unpasteurised milk have been attributed to *Campylobacter*. Poultry products in particular, are considered the main source of infection and contamination by *Campylobacter* in humans. The avian intestinal tract provides a natural environment for *Campylobacter* spp. and colonized broilers could carry around 10^6 to 10^8 cfu/g of *Campylobacter* in their ceca. Therefore, the chicken infection model represents the most suitable *in vivo* model to study *Campylobacter* virulence and pathogenesis particularly in the intestinal environment. CorA is a 37-kDa integral membrane protein that forms the primary constitutive magnesium (Mg^{2+}) uptake system in many bacteria and a knock out mutant deficient in CorA has been previously constructed. In this work, the ability of *C. jejuni* 11168 wild type and *corA* mutant strains to colonize the intestinal tract of a-day-old chick was observed. The chicks were orally infected with *C. jejuni* 11168 wild type and *corA* strains and at 5 and 14 days post infection, *C. jejuni* in the ceca were enumerated. We have observed that the *corA* mutant strains colonized the chicken gut at least 10-fold higher than the wild type strain which indicates that this gene might not be directly involved in intestinal colonisation, and instead, since one group of the chicks was given oral magnesium supplementation, this might suggest that the mutant harbours regulatory systems that confer protection inside the avian host.

P31. Clonal distribution and virulence properties of Campylobacter jejuni blood isolates: implications of ST-677 clonal complex as a major pathogen

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Aims: Campylobacter jejuni is a highly diverse and common enteropathogen, which sometimes causes bacteremia. Our aim was to to detect specific bacterial characteristics which enable C. jejuni to cause bacteremia. Methods: A total of 73 C. jejuni blood isolates, consecutively collected during a 10-year period in Finland, were included. The clinical patient information from these episodes of C. jejuni bacteremia was analyzed. Multilocus sequence typing, lipooligosaccharide locus classification, and detection of putative virulence factor genes ceuE, ciaB, cj0486, and virB11 were performed for the C. jejuni isolates. The isolates were tested for γ-glutamyl transpeptidase production and serum resistance. Major findings: A striking clustering with regard to multilocus sequence typing clonal complexes was detected as 48% of the blood isolates were of the ST-677 clonal complex, an otherwise uncommon complex. Furthermore, although resistance to normal human serum varied among the blood isolates, the isolates of the ST-677 clonal complex were significantly more serum resistant than all other isolates. Sialylated lipooligosaccharide was detected in only 23% of the isolates; it was not associated with serum resistance but was associated with isolates from patients with significant underlying diseases. Main conclusion: Isolates of the ST-677 clonal complex are associated with C. jejuni bacteremia. Impact of the research: The identification of a certain genetic group of C. jejuni which seems to be associated with bacteremia in humans is a novel and important finding. Whether the isolates of the ST-677 clonal complex are specifically adapted to survive in the human bloodstream needs to be studied further.

P32. Successful isolation of *Helicobacter bilis* and *Campylobacter concisus* from a patient with X-linked agammaglobulinemia (XLA).

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Helicobacter bilis (formerly *Flexispira rappini*, then *Helicobacter* sp. flexispira taxon group 8) is an uncommon clinical isolate. An X-linked agammaglobulinemia (XLA) patient with chronic osteomyelitis was diagnosed 15 years ago with *Flexispira rappini* isolated from blood. He has received carbapenems, doxycycline, azithromycin, gentamicin, ciprofloxacin, and fresh

frozen plasma, with associated peripheral neuropathy and temporary hearing loss. Monitoring of CBC, ESR, CRP, and radiologic imaging are used because the *Helicobacter* was not reliably isolated, even when Gram stain positive, due to available culture conditions. With worsening bone scans after a period off therapy, bone, blood, and stool specimens were evaluated at the CDC. Blood cultures were held for five days prior to subculture onto either brain heart infusion agar with 5% rabbit blood or columbia with 10% horse blood. Stool and the bone chip specimens were direct plated onto CVA and the stool also cultured by filtration using non-selective blood medium. All media were incubated at 37°C in a microaerobic environment with increased hydrogen. Phenotypic and molecular methods allowed identification to the species level. *Helicobacter bilis*, resistant to all oral and many IV drugs, was isolated from the blood; two strains of *Campylobacter concisus*, both susceptible to a number of IV and oral agents, were isolated from the bone chip and stool. We isolated two fastidious organisms from a chronic infection, identifying one previously undiagnosed in the patient. Fastidious organisms may cause prolonged and severe infections but remain undiagnosed without appropriate culture techniques, as happened in this case.

P33. Pathogenic Properties of Enterohepatic *Helicobacter* spp. Associated with Intestinal Adenocarcinoma in Rhesus Macaques

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The roles of enterohepatic *Helicobacter* spp. (EHS) in carcinogenesis and their zoonotic or pathogenic potential is being actively studied. We determined the prevalence of EHS infection in a cohort of geriatric rhesus monkeys in which intestinal adenocarcinoma (IAC) is common and investigated the association between EHS infection and IAC. The cohort consisted of 36 animals, 14 of which (age 26–35 years) had IAC. Of the 36 rhesus, 35 (97%) were positive for EHS using PCR or bacterial isolation from feces, colonic or tumor tissues. 16S rRNA gene sequencing revealed that EHS identified in these rhesus monkeys are related to, but distinct from a human clinical isolate *H. fennelliae* CCUG 18820, *H. macacae,* and *H.* sp. MIT 99-5507 Rhesus monkey 2. Thirteen of 14 monkeys with IAC were positive for either *H. macacae* (7/13 (54%)), EHS similar to *H. fennelliae* CCUG 18820 (4/13 (31%)) or a mixture of the two EHS (2/13 (15%)). These results indicate that EHS is prevalent among aged rhesus macaques with IAC and reveal an association between EHS and IAC. Using *Helicobacter*-family specific florescence *in situ* hybridization, EHS could be detected on the surface of colonic epithelia of infected monkeys. All *Helicobacter* isolates, including *H. macacae*, effectively adhered to, invaded, and significantly induced proinflammatory gene expression, including *IL8*, *IL6*, *TNF-α, and iNOS*, while down regulating genes involved in the function of inflammasomes, particularly *IL-1β, NRLP3, and NLRP6* in the human colonic T84 cell line ($p < 0.0001$). These results suggest that EHS may represent an etiological.

P34. *Helicobacter canis* Colonization in Sheep: A Zoonotic Link

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Enterohepatic *Helicobacter spp.* (formerly classified as *Flexispira rappini*) have been identified in sheep and associated with abortion. Because sheep-origin *Helicobacter spp.* have not been extensively characterized, a fecal culture-based survey of several New England sheep flocks was undertaken. Sheep feces were collected in sterile *Brucella* broth containing 10% glycerol. Samples were plated on 5% sheep blood agar and CVA agar and cultured at 37 °C under microaerobic conditions. *Helicobacter*-positive samples were identified by colony morphology, phase contrast microscopy, Gram-negative staining, and *Helicobacter* genus-specific 16S rRNA PCR. *Helicobacter spp.* were cultured from 6 of 23 (26%) sheep from one flock, and their putative species identity and clonality was confirmed by RFLP and REP-PCR. DNA was subsequently extracted from 5 pure isolates for 16S rRNA sequencing. BLASTn alignment confirmed that these isolates shared 99% identity with *Helicobacter canis*. Isolates were subjected to biochemical testing and compared to *H. canis* strains NCTC 12740 (human-origin), ATCC 51401 (dog-origin), MIT 98-153 (cat-origin) and MIT 99-7633 (rhesus macaque-origin). *H. canis* has been associated with canine hepatitis, canine and feline diarrhea, and feline intestinal adenocarcinoma. *H. canis* has also been isolated from humans with bacteremia or gastroenteritis and has been associated with Crohn's disease and hepatitis. In all human cases, patients had a history of dog or cat contact, suggesting zoonotic transmission. This study identifies sheep as a new and potentially important *H. canis* reservoir host suggesting either direct zoonotic transmission or indirect transmission. Improperly processed lamb could also be a source of infection.

P35. *Helicobacter sanguini,* a novel helicobacter isolated from a colony of cotton-top tamarins with a high incidence of ulcerative colitis and colon carcinoma

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Cotton-top tamarins (CTTs: *Saguinus oedipus)* are New World primates native to the rain forests of Colombia. Approximately 50% of colony-maintained animals develop active colitis, with the disease in 25 to 40% of those with active colitis progressing to colonic adenocarcinoma. We first described a novel *Helicobacter* species from cotton-top tamarins in 1999 (Saunders *et al.*, J Clin Micro, 1999). Since then we have surveyed the colony in 2006 and isolated additional strains of this novel *Helicobacter* sp. Full 16S rRNA sequences from six isolates, three from the 1997 study and three from the 2006 survey, indicate that the species is novel with its nearest neighbor being *H. macacae* and *H. trogontum.* Analysis of the hsp60 gene confirms its classification as a novel *Helicobacter sp.* The bacterium grows under microaerobic conditions at 37° and 42° C, but not 25° C, is positive for oxidase and catalase, but does not hydrolyze urea, indoxyl acetate, or alkaline phosphatase. The organism is resistant to nalidixic acid (30 μg disc) and cephalothin (30 μg disc). By electron microscopy, *H. sanguini* has a fusiform morphology and possesses periplasmic fibers and has 6–12 bipolar sheathed flagella. The type strain of *H. sanguini* is MIT 97-6194. The pathogenic potential and identification of virulence properties in *H. sanguini* await further studies.

P36. Use of microarrays to identify phenotypic variation in specific sequence types of *Campylobacter jejuni*

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Campylobacter jejuni sequence type 474 (ST-474) accounted for an estimated quarter of notified campylobacteriosis human cases in New Zealand between 2005 and 2007, but has been rarely found anywhere else in the world. It was the predominant ST in chicken, but was also occasionally found in red meat sources. A recent study comparing two ST-474 isolates discovered an insertion in one of the strains between ORFs Cj1069-Cj1070 with >99% identity to *ykg*C (pyridine nucleotidedisulfide-oxidoreductase protein). An additional study comparing a broad range of ruminant-associated *C.jejuni* strains found the same insertion in different STs. We examined relationships between phenotype and genotype of strains with and without the *ykg*C gene, using the Biolog phenotypic microarray system (Omnilog), exploring differences in the utilisation of carbon, sulphur and phosphorus sources, and tolerance to osmolytes. A variety of human, poultry and ruminant isolates were assayed at two temperatures (37°C and 42°C, simulating mammalian, and poultry guts respectively) for 48h in four different types of plates. In conjunction with KEGG and NCBI the enzymes responsible for the utilisation of the wells have been identified and mapped onto metabolic pathways. Within the 29 identified genes associated with utilisation of the carbon sources, different patterns have been observed between the human, chicken and ruminant isolates. One example is the ability of *C.jejuni* to utilise L-serine which has been reported as being associated with pathogenicity, but serine utilisation was only found in one of the two poultry associated ST-474 isolates. No association between the phenotype and *ykg*C was observed.

P37. Predicting the *Campylobacter jejuni* N-Linked Glycoproteome

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N-linked protein glycosylation is a posttranslational modification involving covalent linkage of an oligosaccharide to an asparagine residue. *Campylobacter jejuni* encodes the best-studied bacterial N-linked protein glycosylation system, in which a heptasaccharide is transferred by the single-subunit oligosaccharyltransferase PglB to an asparagine within the sequon D/E-X-N-X-S/T (X ¹ P). Recent studies suggest a role for *C. jejuni* N-linked protein glycosylation in host-cell attachment/invasion, protease susceptibility and DNA uptake. One of the most interesting aspects of *C. jejuni* N-linked protein glycosylation is that a large number of functionally diverse surface proteins are glycosylated. The objective of this work was to better understand the extent of *C. jejuni* N-linked protein glycosylation by determining the total set of N-linked glycoproteins or N-glycoproteome. Thus, we developed an *in silico* method to identify, from the total predicted proteome, all secreted or transmembrane proteins containing the N-linked glycosylation sequon. This we consider the predicted N-glycoproteome.

Of the 54 experimentally verified *C. jejuni* N-linked glycoproteins this approach identified 51, indicating a robust methodology. Interestingly, we show that the known N-glycoproteins constitute less than half of the total predicted *C. jejuni* N-glycoproteome with a further 55 predicted but not experimentally verified. To validate these results the glycosylation status of predicted but previously unidentified N-glycoproteins was tested experimentally. This work can be easily applied to identify N-glycoproteomes from diverse *Campylobacter* and *Helicobacter* species, enabling their detailed comparison. Identification of the complete N-glycoproteomes will assist with understanding the key question of why many proteins are modified in this way.

P38. *Helicobacter hepaticus* cholesterol-a-glucosyltransferase is integral for establishing colonization in A/JCr mice

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Helicobacter pylori cholesterol-a-glucosyltransferase (*cgt*) catalyzes conversion of cholesterol into cholesteryl-a-glucoside and was shown to be essential for in vivo survival of *H. pylori* in C57BL/ mice. Enterohepatic *Helicobacter hepaticus* contains an ortholog (Hh0676) of the *H. pylori cgt*. To investigate a role of *cgt* in the pathogenesis of *H. hepaticus*, we generated two *cgt*-deficient isogenic mutants (*HhcgtDcat1*, *HhcgtDcat2*) of wild-type *H. hepaticus* 3B1 (Hh) and experimentally inoculated 4 to 6-week-old male A/JCr mice. Cecal and hepatic colonization levels of the Hh mutants and Hh were measured by real-time quantitative PCR (qPCR) at 4 months post-inoculation. Both Hh mutants were undetectable in the cecum of any inoculated mice (10 per mutant) but were detected in two livers (one for each mutant); by contrast, 9 and 7 of 10 mice inoculated with Hh were qPCR-positive in the ceca and livers, respectively. In addition, the mice inoculated with the Hh mutants developed significantly less severe hepatic inflammation ($P < 0.05$) and also produced significantly lower hepatic mRNA levels of proinflammatory cytokines Ifn-g ($P < 0.01$) and Tnf-a ($P \leq 0.02$) as well as anti-inflammatory factors Il10 and Foxp3 compared to the WT 3B1-infected mice. Furthermore, the WT 3B1-infected mice developed significantly higher Th1-associated IgG2a ($P < 0.0001$) and Th2-associated IgG1 responses ($P < 0.0001$) to Hh infection than mice dosed with the Hh mutants. Thus, our data indicate that the cholesterol-a-glucosyltransferase is required for establishing colonization of intestine and liver and therefore is critical for the pathogenesis of *H. hepaticus*.

P39. *Helicobacter pylori* induces semi-maturation of Dendritic Cells through IL-10-mediated STAT3 activation

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Helicobacter pylori colonizes the human stomach and damages the gastric epithelium. Dendritic cells (DCs) regulate the adaptive T cell-mediated immune response, which fails to eliminate the bacterium allowing lifelong persistence. Here, the effect of *H. pylori* on maturation and activation of human DCs was investigated. Human monocyte-derived DCs (MoDCs) were infected with *H. pylori* and the expression of the activation marker CD83 and the co-stimulatory molecules CD80/CD86 were analyzed. MoDCs primed with live *H. pylori* expressed lower levels of CD83 and CD80/CD86 compared to MoDCs stimulated with PFA-fixed *Helicobacter*, suggesting that bacterial virulence factors were involved. Infection of MoDCs with several *H. pylori* mutant strains revealed that CagA predominantly repressed DC maturation, and CagA was responsible for a strong secretion of anti-inflammatory IL-10, leading to an expansion of Foxp3-expressing regulatory T cells (Tregs) over Th1 cells. Depletion of STAT3 in DCs has been described to be involved in impaired mucosal tolerance in mice. Therefore, activation of STAT3 was evaluated in MoDCs after *H. pylori* infection. High levels of phosphorylated STAT3 were determined in MoDCs upon infection with wild type *H. pylori*, while CagA-deficient *Helicobacter* caused lower STAT3 activation. IL-10 was shown to be responsible for STAT3 activation, since IL-10 neutralization lead to a block of STAT3 phosphorylation and restored DC maturation, which in turn induced an increase of IFN-γ-producing T cells and reduced the amount of Tregs. Together, our results show a novel mechanism by which *H. pylori* modulates maturation of human DCs and the subsequent immune response.

P40. Relevance of Dsb system for protein activity: a case of alkaline phosphatase PhoX from Campylobacter jejuni

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Background: The Dsb (disulfide bond) system contributes to the correct folding of numerous bacterial extracytoplasmic proteins. Disulfide bridges, introduced to substrate proteins in Dsb oxidative pathway, ensure the stability of their tertiary and quaternary structures. The Campylobacter jejuni Dsb system comprises four enzymes: two putative periplasmic disulfide donor proteins DsbA1 and DsbA2 and two membrane oxidative proteins DsbB and DsbI. Here we show the impact of oxidative folding on protein activity on the example of Dsb substrate, alkaline phosphatase PhoX, which releases Pi from organic phosphomonoesters. Methods: C. jejuni dsb and phoX knock-out mutants were constructed by allele exchange. Point mutated versions of cjphoX were generated by means of site-directed mutagenesis (QIAGEN) and introduced to C. jejuni phoX knock-out cells by conjugation. PhoX enzymatic assays were performed with the use of pNPP (4-Nitrophenyl phosphate disodium salt). Major Findings: Examination of PhoX activity in the C. jejuni dsb mutants (dsbA1/A2/B/I) comparing to WT strain documented the relevance of respective CjDsb for ensuring PhoX functionality. Complementation of the C. jejuni phoX mutant with different plasmids expressing a PhoX variants (one of the five cysteines replaced by alanine) revealed that the 2nd and 4th cysteine residue in CjPhoX protein are connected by a disulfide bridge, crucial for enzyme activity. Impact Of The Research: Our findings demonstrate the relevance of oxidative folding for achieving activity of bacterial extracytoplasmic proteins. Given that some of them constitute virulence factors, proper functioning of Dsb system has an innegligible role for bacterial virulence.

P41. Variation in the distribution of the MarR-type transcriptional regulators Cj1546 and Cj1556 amongst *Campylobacter jejuni* strains correlates with differences in resistance to oxidative and aerobic stress

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The ability of *Campylobacter jejuni* to respond to oxidative stresses is believed to be paramount during survival in the environment and *in vivo*. The re-annotation of the *C. jejuni* NCTC11168 genome sequence led to the identification of both Cj1546 and Cj1556 as MarR-type transcriptional regulators. We have previously shown that a *C. jejuni* 11168H *Cj1556* mutant exhibited increased sensitivity to both oxidative and aerobic stress, decreased ability for intracellular survival in Caco-2 human intestinal epithelial cells and J774A.1 mouse macrophages and a reduction in virulence in the *Galleria mellonella* infection model. Microarray analysis of gene expression changes in the *Cj1556* mutant indicated negative autoregulation of *Cj1556* expression and down-regulation of genes associated with oxidative and aerobic stress responses. Further studies have shown that a 11168H *Cj1546* mutant also exhibits increased sensitivity to oxidative and aerobic stress. However there are differences in the distribution of *Cj1546* and *Cj1556* amongst *C. jejuni* wild-type strains, with livestock clade strains containing both *Cj1546* and *Cj1556*, whilst non-livestock clade strains contain only *Cj1546*. Non-livestock strains appear to be more resistant to oxidative stress than livestock strains. The effect of a *Cj1546* mutation is more pronounced in livestock strains such as 11168H and 81–176 than in non-livestock strains such as 81116. These studies indicate a role for both Cj1546 and Cj1556 in the *C. jejuni* oxidative and aerobic stress responses, yet also suggest some divergence in the ability to respond to such stresses between different *C. jejuni* wild-type strains.

P42. *Campylobacter jejuni* gene polymorphism: a determinant for the development of Guillain-Barré syndrome in Bangladesh

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Background: *Campylobacter jejuni* is the most frequently identified preceding infection in Guillain-Barré syndrome (GBS) and Miller Fisher variant (MFS) . Molecular mimicry between *C. jejuni* lipo-oligosaccharides (LOS) and peripheral nerve gangliosides plays a crucial role in the pathogenesis of the GBS. In this study, we determined the genetic polymorphism involved in synthesis of LOS in *C. jejuni*, which in turn determines autoantibody reactivity, and clinical phenotype of GBS and MFS. Methods: *C. jejuni* strains were isolated from 59 patients with GBS/MFS (n=20) and enteritis (n=39) in Bangladesh. We determined the frequency of *cst-II* gene and polymorphism (Asn/Thr51) in relation with the bacterial ganglioside epitopes, and autoantibody reactivity. Results: A1 sub-class was the most common sub-class among LOS class A strains (8/10, 80%) from GBS patients. *C. jejuni cst-II* (Thr51) was significantly present in GBS-related strains than did enteritis ones (80% vs 5%; $p< 0.001$). Patients who had *C. jejuni* (*cst-II,* Thr51) were positive for anti-GM1 antibody (90%) and anti-GD1a IgG (30%), and all patients had limb weakness. Strains with *cst-II* (Thr51) frequently expressed the GM1/GD1 epitope (80%), whereas those with *cst-II* (Asn51) had the GA2 and GD3 epitopes. All patients infected with *C. jejuni* (Asn51) had anti-GQ1b antibody response, and had ophthalmoparesis and ataxia. Conclusions: This is the first report demonstrating *cst-II* polymorphism in GBS related *C. jejuni;* isolates in Bangladesh. Our results support the hypothesis that genetic polymorphism of *C. jejuni* modifies the substrate specificity of LOS biosynthesis enzyme and that autoantibody reactivity determines the clinical presentation of GBS.

P43. Role of a putative *cheZ* orthologue in *Campylobacter jejuni* chemotaxis

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The major food borne pathogen *Campylobacter jejuni*, utilizes chemotaxis to colonise the chicken gastrointestine. Similar to the *Escherichia coli* chemotaxis system, *C. jejuni* produces the CheA-CheW-CheY signal transduction backbone that transduces, by phospo-relay, signals from surface chemoreceptors to modulate flagellar rotation. In contrast to *E. coli*, *C. jejuni* also expresses a CheV, a CheB missing a response regulator (RR), and its CheA contains an additional RR domain. The level of phosphorylated CheY is regulated in *E. coli* by the phosphatase, CheZ, however, until recently *C. jejuni* was thought to lack a CheZ homologue. The discovery of the CheZ orthologue, HP0170, in *Helicobacter pylori* led to identification of Cj0700, a putative *Campylobacter* CheZ orthologue. The aim of this protect is to investigate role of *cj0700* in *C. jejuni* chemotaxis. A mutant (Δ*cj0700)* and the cognate complement (Δ*cj0700, Cj0046::cj0700*) were constructed in *C.jejuni* NCTC 11168. No growth differences were seen between Δ*cj0700*, *ΔcheY* and wild-type. On semi-solid agar the Δ*cj0700* mutant strain showed reduced motility relative to the wild-type and this phenotype was reversed in the complement. Cj0700 was expressed as a His-tagged protein and found to dephosphorylate *C. jejuni* CheY. Expressed Cj0700 also dephosphorylated CheA-RR-P and CheV-P, but less efficiently than CheY-P. Our findings indicate that *cj0700* has a role in *C.jejuni* chemotaxis and as Cj0700 can promote dephosphorylation of CheY, it is likely to be a CheZ orthologue.

P44. Presence of virulence genes, adhesion and invasion of *Arcobacter butzleri*

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The pathogenic potential of *Arcobacter butzleri* isolates was investigated by detecting the presence of putative virulence genes and analysing the adhesive and invasive capabilities in cell cultures of human cell lines. The presence of 10 putative virulence

genes in 52 *A. butzleri* isolates was determined by PCR. The genes *ciaB*, *mviN*, *pldA*, *tlyA*, *cj1349* and *cadF* were detected in all, while *irgA* (15 %), *iroE* (60 %), *hecB* (44 %) and *hecA* (13 %) were detected only in few *A. butzleri* isolates. On HT-29 cells, four out of six isolates adhered and three of them were able to invade, while all six isolates adhered and invaded Caco-2 cells with higher degrees. The genes *ciaB*, *cadF* and *cj1349* of all six isolates were sequenced but no considerable changes of the amino acids in putative functional domains were observed. We conclude that selected *A. butzleri* isolates adhere to and invade various human cell lines, which emphasize their human pathogenic potential. The efficiency of invasion depends on the eukaryotic cell line and the individual bacterial strain used. We could not show any functional correlation between the amino acid sequence of CadF, CiaB or Cj1349 and the adhesive or invasive phenotype.

P45. The methylmenaquinol:fumarate reductase (Mfr) contributes to *Campylobacter jejuni*'s resistance to hydrogen peroxide and persistence in murine macrophages

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The methylmenaquinol:fumarate reductase (Mfr) of *Campylobacter jejuni* is a periplasmic respiratory (redox) protein that contributes to the metabolism of fumarate and displays homology to succinate dehydrogenase (Sdh). Since chemically oxidized redox-enzymes, including fumarate reductase and Sdh, contribute to the generation of oxidative stress in *Escherichia coli*, we assessed the role of Mfr in *C. jejuni*'s resistance to hydrogen peroxide (H_2O_2). Our results show that an Mfr deletion mutant (Δ*mfrA*) was less susceptible to H_2O_2 as compared to the wildtype (WT); however, both strains exhibited a similar phenotype when challenged with organic peroxides or paraquat. The catalase activity was lower in the Δ*mfrA*, while the H_2O_2 concentrations in the mutant cells and broth cultures were significantly higher than that of WT after exposure to the oxidant. Further, H_2O_2 resulted in a significant decrease in free intracellular iron in the Δ*mfrA* as compared to WT, while the addition of iron reduced both the Δ*mfrA*'s resistance to H_2O_2 and the accumulation of the oxidant in the mutant's growth medium. The Δ*mfrA* was impaired in adherence to and invasion of human INT-407 cells but not in intracellular survival, while the mutant was significantly more persistent in RAW macrophages as compared to the WT. Scanning electron microscopy showed that infection with the Δ*mfrA* resulted in prolonged changes to the macrophages' morphology, mainly a formation of spherical-shaped cells replete with budding structures and craters. Collectively, our results suggest a role for Mfr in *C. jejuni*'s resistance to H_2O_2-associated stress.

P46. Modulation of Campylobacter jejuni Pathogenicity as a Consequence of Environmental Stress Response

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Introduction: Campylobacters have developed a number of mechanisms for responding to environmental conditions. However, the different virulence properties of these cells following exposure to stress are still poorly understood. Methods: We analysed in vitro response to environmental stress (starvation, oxidative stress, heat shock) and the consequent modulation of Campylobacter jejuni pathogenicity in eukaryotic cell (Caco-2, J774 murine macrophages) and in vivo in a murine model. Results: In vitro, the influence of starvation and oxidative stress has milder effect than that of heat shock, although all of the stress conditions influenced the survival of C. jejuni. As published, we proved that environmental stresses have influenced the adhesion, invasion and intraepithelial survival of C. jejuni in eukaryotic cell models as well as the course of the infection of BALB/c mice. The systemic infection of mice occurred no matter what stress was investigated, but with different bacterial load in their livers and spleens as well as different production dynamics of the cytokines investigated (interleukins 6 and 10, tumor necrosis factor-α, interferon-γ) in plasma and liver homogenates. The most pronounced differences were in interferon-γ and interleukin 10 productions, indicating their roles in the immune response to C. jejuni infection. Impact of research: The study of environmental impact on bacterial virulence reveals that microbial adaptation during stress challenge is crucial not just for pathogen survival out of the host, but also during host-pathogen interactions, and thus for the bacterial pathogenicity.

P47. Mobility of DNA sequence recognition domains in DNA methyltransferases suggests epigenetics-driven adaptive evolution

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DNA methylation is one of the best studied epigenetic modifications observed in prokaryotes. It affects nearby gene expression. Most DNA methylation reactions in prokaryotes are catalyzed by a DNA methyltransferase, especially the modification enzyme of a restriction-modification system. Its target recognition domain recognizes a specific DNA sequence for methylation. Through comparison of complete genome sequences of global *H. pylori* strains at the single bp resolution, we obtained evidence for movement of target recognition domains between non-orthologous genes and their movement within a gene. These are likely mediated by DNA recombination machinery and are expected to alter the methylation status of a genome. Such alterations potentially lead to changes in global gene expression pattern and various phenotypes. The targets of natural selection in adaptive evolution might be these diverse methylomes rather than diverse genome sequences, the targets according to the current paradigm in biology. This "epigenetics-driven adaptive evolution" hypothesis can explain several observations in the evolution of prokaryotes. Impact of the research. The *H. pylori* oxidizing Dsb system is a novel and different from the Dsb system in *E. coli.*

P48. *Helicobacter pylori* HPO377, a member of a Dsb family, is potentially involved in cytochrome c maturation

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Aims: In gramnegative bacteria, the oxidative protein folding takes place in the periplasm and is controlled by proteins from the Dsb family (disulfide bonds). However, in the periplasm, there is also a need for selected proteins to be kept in a reduced form. Assembly of c-type cytochromes, essential for energy metabolism, is a case of point. The aim of the work presented here was to characterise *H. pylori* potential oxidoreductase - HP0377 containing TRX fold with CXXC motif. Methods: The studies made use a combination of biochemical, standard molecular biology and bioinformatic procedures. Major findings: The conducted fold-recognition analyses showed that HP0377 described as DsbC homologue is rather a counterpart of CcmG/DsbE, which plays a role in c-type cytochrome maturation. Despite numerous attempts, we were unable to construct a *hp0377* knock-out mutant, which indicates that HP0377 may be an essential protein, in contrast to EcCcmG. HP0377 does not complement *E. coli dsbA* mutation and is inactive in insulin reduction test. Its redox potential is -171 mV. The pKa values of the two active site cystein thiol groups are different as pKa plot showed two transistions. HP0377 is present in the redox form in *H. pylori* cells and in oxidize form in *E. coli* cells. Main conclusion: Probably HP0377, which does not cooperate with EcDsbD, plays an essential role in *Helicobacter pylori*. Impact of the research: Our results showed that HP0377 is most likely an untypical counterpart of CcmG and expanded our knowledge about novel Dsb system.

P49. Characterisation of a Multi-ligand Binding Chemoreceptor *CcmL* (Tlp3) of *Campylobacter jejuni*

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Campylobacter jejuni is the leading cause of human gastroenteritis worldwide with over 500 million cases annually. Chemotaxis and motility have been identified as important virulence factors associated with *C. jejuni* colonisation. Aims: To characterise group A transducer like protein Tlp3 and the ligands it can bind, while sensing the external environment for bacterial movement to or away from a chemical gradient or stimulus. Methods: This study shows Tlp3 (cj1564) to be a multi-ligand binding chemoreceptor and reports direct evidence supporting its involvement in the chemotaxis signalling pathway via

small molecule arrays, SPR, NMR and chemotaxis assays. Major findings: We further demonstrate its ability to interact with both chemoattractants (isoleucine, purine, malic acid and fumaric acid) and chemorepellents (lysine, glucosamine, succinic acid, arginine and thiamine). An isogenic mutant of *tlp3* was shown to alter phenotypic characteristics of *C. jejuni*, encompassing bacterial cell shape, motility, autoagglutination behaviour and biofilm formation. Additionally, protein-protein interaction studies revealed signal transduction initiation through the scaffolding protein CheV in the chemotaxis sensory pathway. We demonstrate Tlp3 to have a role in invasion as *in vitro* assays the *tlp3* isogenic mutant has reduced ability to adhere and invade cultured epithelial cell line; interestingly however colonisation ability of avian caeca appears to be unaltered. Conclusion: This is the first report characterising Tlp3 as a multi-ligand receptor for *Campylobacter jejuni* (CcmL), *Campylobacter* chemoreceptor for multiple ligands.

P50. Mechanisms of pinocembrin anti-*Campylobacter* activity

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Campylobacteriosis is a leading bacterial food-borne disease worldwide facing increasing resistance against several clinical antibiotics. Therefore, new effective natural antimicrobials are needed for application in medicine and/or antimicrobial prevention in food production and supply chains. One of the active natural compounds is pinocembrin – a flavonoid compound with various bioactivities, found in the extracts of some medicinal plants (e.g. *Alpinia katsumadai)*. We investigated anti-*Campylobacter* activity and the dose-dependent mechanisms of pinocembrin action. For this purpose the minimal inhibitory concentration (MIC=64 μg/ml) was determined by broth microdilution and further tested also in subinhibitory (0.25 MIC) and suprainhibitory (2 MIC) concentrations. We studied the influence of subinhibitory concentration of pinocembrin on gene expression in the *C. jejuni* NCTC 11168 mutant of the *cmeR* transcriptional repressor by microarray analysis and confirmed the results with qRT-PCR. Further, we measured the physiological influence of pinocembrin (between subinhibitory and suprainhibitory values) on time-kill kinetics (broth macrodilution), on intracellular oxidation evaluated by dihydrodichlorofluorescein diacetate and on membrane integrity by following viability (Bacterial Viability Kit LIVE/DEAD *Bac*Light). The results on molecular level show upregulated expression of several motility-related genes, as well as genes involved in redox signalling and antioxidant defence. Influence of pinocembrin on antioxidant defence was reconfirmed phenotypically, where it worked as an antioxidant and promoted bacterial growth in subinhibitory concentrations. However, MIC significantly decreased viability of cells, whereas suprainhibitory concentration worked bactericidally and it severely affected the membrane integrity. Effect of pinocembrin is dose-dependent, therefore the concentration for its application needs to be carefully chosen.

P51. Clonally related *Campylobacter coli* isolates of human and poultry origin differ in cytotoxicity and posttranslational processing of the major outer membrane protein PorA

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Knowledge of pathogenic determinants of *Campylobacter jejuni* and *Campylobacter coli* and their association with different genotypes are still largely unclear. As part of a larger study to identify pathogenic determinants in *Campylobacter* species of zoonotic potential we screened isolates from chicken and human clinical cases for cytotoxicity to human intestinal cell lines. Two strains of *C. coli* with identical MLST and *fla* genotypes that differ in host origin and degree of cytotoxicity were identified and compared on the proteome level by 2-D gel electrophoresis using different pH gradients followed by a combination of MALDI-TOF-MS/MS and LC-MS/MS. Both strains produced almost identical protein profiles confirming their strong clonal relatedness. However, the cytotoxic human isolate displayed a cluster of spots that were absent in the non-cytotoxic chicken isolate. MS analysis of tryptic digests of these spots showed that they were generated by extensive processing of the major outer membrane protein PorA. PorA is a highly expressed and variable outer membrane protein

that is recognised by the immune response during campylobacterosis. Our results suggest an alternative mechanism to antigenic variability by which processing of PorA may influence how *C. coli* interacts with its environment.

P52. *Helicobacter pylori* protein JHP0290 induces TNF-a release and apoptosis in macrophages

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Helicobacter pylori infection induces local inflammation in the stomach associated with induction of proinflammatory cytokines and apoptosis. Activated macrophages at the sub-mucosal space play a major role in generating innate immune response against *H. pylori*. Previous studies have shown that JHP0290, a highly conserved secreted protein of unknown function from *H. pylori*, is upregulated during acidic stress. JHP0290 is one of the antigens, which is preferentially recognized by the sera of *H. pylori* infected patients and a study suggested that JHP0290 is one of the potential biomarker for gastric cancer in China. Our study shows that recombinant purified JHP0290 induces the release of proinflammatory cytokine Tumor Necrosis Factor-a (TNF-a) and apoptosis in macrophages. A mutant strain of *H. pylori* disrupted in *jhp0290* gene was significantly impaired in its ability to induce TNF-a and apoptosis. Recombinant JHP0290 binds to macrophages and human gastric epithelial cell line AGS, suggesting the involvement of certain cell surface receptor in recognition of this protein. Intracellular signaling involving Src family of tyrosine kinases and ERK MAPK are required for JHP0290-induced TNF-a release from macrophages. Furthermore, JHP0290 induced TNF-a release is partly dependent of activation of transcription factor NFkB. These results point to the putative role of JHP0290 in chronic gastric inflammation and therefore, possible role in *H. pylori* associated disease development.

P53. Study of the role of gamma-glutamyl transpeptidase of *Campylobacter jejuni* on apoptosis of epithelial cells and lymphocyte proliferation

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15–20% of *Campylobacter jejuni* isolates produce a gamma-glutamyl transpeptidase (GGT) which has 75% homology with *Helicobacter pylori* GGT suggesting a conserved activity. Unlike H. pylori GGT, *C. jejuni* GGT has been the topic of very few studies. In line with data available for *H. pylori*, our aim was to evaluate the inhibitory and proapoptotic activities of C. jejuni GGT on intestinal epithelial cells and human lymphocytes. The activity of culture supernatants of a *C. jejuni* reference strain expressing GGT and its corresponding GGT-knockout was measured on AGS and Caco2 epithelial cell proliferation using a MTS tetrazolium test. Proapoptotic activity and its influence on the lymphocyte cell cycle were studied using the method of Nicoletti et al., based on propidium iodide. The GGT inhibitory action on lymphocyte proliferation was assessed by BrdU incorporation. *C. jejuni* GGT inhibited the proliferation of both epithelial cell lines, but with no proapoptotic activity. The GGT inhibited lymphocyte proliferation by causing cell cycle arrest in the G0/1 phase. These effects were not observed with supernatants of the GGT-KO strain nor after GGT enzymatic inhibition by eating or administering a specific pharmacological GGT inhibitor (acivicin). *C. jejuni* GGT activity is therefore comparable to that of *H. pylori* and more generally to that of Epsilonproteobacteria. It could therefore be considered as a pathogenicity factor, promoting the persistence of the bacteria in the host, via inhibition of lymphocyte proliferation. These observations are also consistent with the role of this enzyme in the pathophysiology of *C. jejuni* associated chronic infections.

P54. Utilization of salmochelins by *Campylobacter jejuni* via ferric enterobactin iron acquisition system

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The high affinity enterobactin(Ent)-mediated iron scavenging is tightly linked to *Campylobacter* pathogenesis. To date, Ent is the only known siderophore produced by enteric microorganisms for *Campylobacter* iron acquisition during infection.

We speculate that *Campylobacter* could utilize structurally diverse catecholates through the FeEnt acquisition system. Of these, salmochelins, the glucosylated derivatives of Ent, are of particular interest because they could confer an *in vivo* growth advantage to enteric bacteria by resisting lipocalin-2, a host acute phase protein with high affinity to Ent. In this study, the *C*-glycosyltrasnferase IroB was purified for *in vitro* synthesis of various salmochelins by glucosylation of Ent, which include mono, di- and triglucosyl Ent (MGE, DGE, and TGE, respectively). A quantitative, microtiter plate-based growth promotion assay indicated that *C. jejuni* ATCC 33560 could utilize these salmochelins in a CfrB receptor-dependent manner. The periplasmic trilactone esterase Cee also played an essential role in the salmochelin-mediated iron acquisition, consistent with the finding that Cee could hydrolyze salmochelins *in vitro*. Interestingly, NCTC 11168 failed to utilize DGE and TGE although it could efficiently utilize MGE via functional CfrA, indicating the FeEnt receptor CfrA and CfrB display different substrate specificity for salmochelin-mediated iron acquisition. Together, this study firmly established that *Campylobacter* could utilize high-affinity salmochelin for iron acquisition, and provided insights into the delicate interaction between *Campylobacter* and host during infection.

P55. Abundance and diversity of N-linked protein glycosylation systems within the Epsilon Proteobacteria

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The Campylobacter jejuni N-linked protein glycosylation system involves glycosyltransferase-mediated assembly of a lipid-linked heptasaccharide on the cytoplasmic face of the inner membrane and, following 'flipping' into the periplasm, oligosaccharyltransferase-mediated transfer of glycan onto an extended D/E-X-N-X-S/T sequon present on a functionally diverse set of surface proteins. The entire N-glycosylation machinery is encoded by a single protein glycosylation (pgl) locus including the pglB gene that encodes the oligosaccharyltransferase. Bacterial genome sequence data indicates that pglB orthologues are found in species from the Delta and particularly Epsilon subdivisions of the Proteobacteria but not in other subdivisions. The associated pgl gene loci vary in arrangement and gene content with some Campylobacter and Helicobacter species containing two pglB genes. The corresponding putative N-linked protein glycosylation systems from these species remain for the most part uncharacterised. We have used a variety of approaches to characterise N-linked protein glycosylation systems in diverse Campylobacter, Helicobacter, Wolinella and environmental Delta/Epsilon Proteobacteria species isolated from hydrothermal vents such as Nautilia profundicola, Sulfurovum lithotrophicum and Deferribacter desulfuricans. Employing in vitro peptide glycosylation assays, insertional knock out mutagenesis and mass spectrometry we have demonstrated N-linked glycan structural diversity and elucidated the role of individual gene products in glycan assembly thereby providing basic models for the roles of Helicobacter and Wolinella pgl genes in assembly of the corresponding N-linked penta- and hexasaccharide glycans. Furthermore genetic complementation experiments to compare activities of the pglB encoded oligosaccharyltransferases from a range of species demonstrated significant differences in activity.

P56. The Effect of Proton Pump Inhibitors on the Survival, Motility And Morphology of *Campylobacter jejuni*

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Introduction: Proton pump inhibitors (PPIs) are primarily used in the treatment of conditions such as peptic ulcer, heartburn and gastroesophageal reflux disease. Due to their effectiveness and safety profile they are one of the most commonly prescribed family of drugs in the world. It is well known that patients being treated with PPIs are more susceptible to enteric infections such as campylobacteriosis. Methods: Broth microdilution was used to determine the minimum bactericidal concentration (MBC) of the PPI pantoprazole for three strains of *C. jejuni*. 0.4% sloppy agar and electron microscopy were used to assess the affect of pantoprazole on *Campylobacter* motility and morphology respectively. The effect of pantoprazole on the transcriptome and proteome of *C. jejuni* was also examined. Results: The median MBC for *C. jejuni* strains 11168, 81116 and 81–176 (pVir+) was found to be 0.83, 0.99 and 1.27 mg/ml respectively. Motility of *C. jejuni* was adversely affected, in a dose dependent manner, following exposure to pantoprazole for 24 hours. Exposure to levels of pantoprazole above the

MBC resulted in coccal forms of *C. jejuni* being generated. Proteomic analysis suggests that PPI exposure may result in oxidative stress and have affects on cell wall biosynthesis and the electron transport chain of *C. jejuni*. Further work will assess the adhesion/invasion capabilities of *C. jejuni* following exposure to PPIs. Impact: There exists an interesting dichotomy whereby people taking PPIs appear to be more susceptible to enteric infections and yet *C. jejuni* is adversely affected by PPIs in vitro.

P57. Alterations of glycosylation induced by Helicobacter pylori during gastric carcinogenesis.

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Helicobacter pylori attachment to human gastric mucosa is mediated by bacterial adhesins, such as BabA, that recognizes histo-blood group antigens expressed by epithelial cells. The gastric glycosylation patterns define H. pylori tropism, and bacteria are mainly found at the surface mucous cells, where there is H-type 1 and Lewis b expression. The colonization of the deeper glands is rare due to the presence of terminal α1,4-linked-N-acetylglucosamine residues, that inhibit the synthesis of a H. pylori cell wall constituent, and therefore suppresses bacterial growth. H. pylori colonization promotes alterations of gastric mucosa glycosylation. Our aim was to identify the molecular mechanisms underlying these modifications. We have demonstrated using cell models that H. pylori infection induces the expression of glycosyltranferases, namely β3GnT5, involved in the biosynthesis of sialylated Lewis antigens, which are receptors for the bacterial sialic acid binding adhesin (SabA). Evaluation of transcript levels of glycosyltransferases and sialylated-Lewis antigens expression in gastric tissue showed that increased expression of sialyl-Lewis a/x observed during chronic infection are accompanied by increased transcript levels of the B3GNT5, B3GALT5 and FUT3 genes. This glycosylation shift results in increased SabA-mediated bacterial attachment to gastric mucosa. Additional glycosylation modifications that occur during gastric carcinogenesis, such as Sialyl-Tn antigen expression in intestinal metaplasia, will also be discussed in the view of their potential application as biomarkers. The identification of the pathways that regulate gastric mucosa glycosylation discloses molecular mechanisms that are crucial for bacteria life-long infection and disease progression.

P58. Comparative genomics of hyper-invasive *Campylobacter jejuni* strains.

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C. jejuni is a major cause of food borne infections in humans. Invasion across the human intestinal epithelium has been studied as a key virulence factor during *C. jejuni* enteritis. The aim of this work was to develop our understanding of *C. jejuni* pathogenesis and how this organism has evolved to become a major human pathogen. To do this a unique group of six hyper-invasive *C. jejuni* strains have been compared at the whole genome level to a panel of low invasive strains using Comparative Genomic Hybridization (CGH), Pooled Suppressive Subtractive Hybridization (PSSH) and more recently Whole Genome Sequencing (WGS). Further analysis of the CGH and PSSH data has identified distinctive loci significantly associated with the hyper-invasive *C. jejuni* strains. Whole genome sequencing of a number of the hyper-invasive strains has also revealed mosaicism in the capsule locus with genes homologous to other *Campylobacter* species. Mutation of one of these genes resulted in reduced invasion in Caco2 cells with no change in serotype suggesting a role for the capsule in this hyper-invasive phenotype, but not as the serodeterminant of the Penner serotyping scheme. Overall we present a comparative genomics study of a group of phenotypically related strains of *C. jejuni* and offer some insight into the role of the capsule in this hyper-invasive phenotype.

P59. Understanding the impact of amino acid catabolism on the intracellular survival capacity of *Campylobacter jejuni*

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The metabolic properties of *Campylobacter jejuni* enabling an effective colonization of its hosts are not well characterized. Sugars are generally not utilized, whereas the amino acids asparagine, aspartate, glutamine, glutamate, proline and serine are ideal growth substrates for *C. jejuni* 81–176 *in vitro* and in a murine infection model. Their utilization is facilitated by transport systems encoded by the *peb1*, *putAP* and the *sdaCA* operons. Inactivation of one of these transporters leads to only slightly proliferation reduction of respective *C. jejuni* 81–176 mutants in nutrient rich medium, suggesting a redundancy in the amino acid mediated growth. Here we examined the influence of amino acid catabolism on the intracellular survival capacity of *C. jejuni* 81–176. To circumvent the problem of redundancy, we constructed in *C. jejuni* 81–176 not only single *peb1*, *putAP* and *sdaCA* deletion mutants, but also double deletion mutants and a triple mutant lacking all three operons with the *Cre-loxP*-system. Their growth was analyzed *in vitro* and changes in the amino acid uptake were monitored by exometabolome analysis. The intracellular survival of the wild-type strain and its isogenic mutants was tested in human colonic T84 cells with the gentamicin protection assay. With this project we have for the first time successfully demonstrated the *in vivo* use of the *Cre-loxP*-system in *C. jejuni*. We have gained new insights into the catabolic properties of *C. jejuni* 81–176 and a better understanding of the metabolic interaction between *C. jejuni* 81–176 and its hosts.

P60. Use of SILAC Quantitative Proteomics for analysis of Campylobacter jejuni protein expression.

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Introduction: The transcriptomic comparison of gene expression is an important part of the arsenal of OMICs technologies, but ideally requires verification at the protein level. Mass spectrometry-based quantitative proteomics can quantify thousands of proteins in complex biological samples, including membrane proteins, but requires adaptation to the specific biological system studied. Here we describe the implementation of quantitative proteomics based on Stable Isotope Labeling with Amino Acids in Culture (SILAC) for use with *Campylobacter jejuni*. Results: The conditions required to apply Stable Isotope Labelling of Amino Acids in Culture (SILAC) for the quantitative analysis of protein expression in Campylobacter were examined using Heavy Arginine ($^{13}C_6$, $^{15}N_4$) and Heavy Lysine ($^{13}C_6$) as the labels. Incorporation studies demonstrated that in the *Campylobacter jejuni* NCTC 11168 wild-type strain, only the Heavy Arginine effectively incorporated (>95%) into the proteins. Heavy Lysine incorporation was only 50%, presumably due to an active Lysine Biosynthesis pathway. This was confirmed when the same incorporation experiment was repeated using a mutant in which the *lysA* (Cj0314) gene was disrupted. The absence of a functional Lysine Biosynthesis pathway was confirmed as the strain required exogenous lysine supplementation for growth. SILAC incorporation studies with the *lysA* mutant showed that all proteins analysed now incorporated both Heavy [AVV1] Lysine and Arginine effectively (>98%). Impact: The availability of SILAC technology for *Campylobacter* research will allow for quantitative proteomics and will support future studies on gene expression in this important pathogen. The ability to use both labels will increase the coverage of quantitation.

P61. Application of SILAC Quantitative Proteomics for analysis of a *Campylobacter jejuni perR-fur*- Mutant.

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Introduction: As a microaerophile, *Campylobacter jejuni* is often exposed to oxidative stress. The PerR and Fur regulatory proteins are well characterised in *Campylobacter*, and repress expression of several proteins including catalase, alkyl hydroperoxide reductase, and the iron uptake systems in response to levels of oxidative stress and iron availability. In this study we have used SILAC-based quantitative proteomics to investigate the changes in protein expression in a *C. jejuni perR fur* mutant. Results: . SILAC was used to compare protein expression between *C. jejuni* NCTC 11168 wild-type and *perR fur*

mutant, using heavy Arginine ($^{13}C_6$, $^{15}N_4$) as the label. Following microaerobic growth at 37°C, the stains were mixed prior to lysis and fractionation into Soluble, Inner Membrane and Outer Membrane Fractions. Each Fraction was run on SDS-PAGE and the whole lane subjected to in-gel Trypsin digestion prior to LC-MS-MS analysis on an Orbitrap-XL. In total 32 fractions from the SDS-PAGE were analysed on the Orbitrap and the data processed using MaxQuant for Identification and Heavy/Light quantitation. A total of 1368 proteins (84% of the total proteome) was detected, with quantification possible for 1064 (66%) proteins, making this the most complete coverage of proteomic data described for *Campylobacter* to date. Over 100 proteins were found to have >2 fold changes in expression in the *perR fur* mutant, and these included catalase (29-fold up); AhpC (5-fold up); and CfrA (8-fold up). Impact: SILAC can be used for quantitative proteomics in *Campylobacter* regulatory mutants, thus expanding the OMICs toolbox available for *Campylobacter* research.

P62. Reinvestigation into the mechanisms of *Campylobacter jejuni* invasion of intestinal epithelial cells

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The ability of *C. jejuni* to invade intestinal epithelial cells (IECs) *in vitro* is well established, but the molecular mechanisms involved are still a matter of controversy, including the role of the bacterial virulence factor CiaB. Other enteric pathogens induce re-arrangement of host cytoskeletal structures, such as microfilaments (MFs) and/or microtubules (MTs), resulting in bacterial internalisation via endocytosis. *C. jejuni* internalisation has been reported to require mainly MF-, mainly MT-, both MF- and MT-dependent mechanisms or neither, resulting in considerable confusion in the literature. We decided to reinvestigate the inhibition of *C. jejuni* invasion of IECs, as well as the role of both CiaB and CadF in bacterial interactions with IECs. Inhibition of caveolar endocyctosis using MbCD decreased *C. jejuni* invasion, whilst inhibition of actin polymerisation using cytochalasin D increased *C. jejuni* invasion. Experiments with other inhibitors of bacterial invasion such as AP180 (clathrin-mediated endocytosis), wortmannin (macropinocytosis) and colchicine (microtubules) are in progress. 11168H *cadF* and *ciaB* mutants exhibited a reduction in both interaction with and invasion of IECs compared to the wild-type strain and reduced virulence in the *Galleria mellonella* larvae model. *C. jejuni* outer membrane vesicles adhere to T84 IECs via a CadF-mediated binding mechanism and OMVs isolated from the *cadF* mutant induce a reduce host innate immune response. Further studies are ongoing to investigate both the mechanisms of *C. jejuni* invasion, the bacterial factors involved and the host innate immune response to *C. jejuni* invasion.

P63. Opsonic activity against *Campylobacter concisus* of serum from infected patients

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Intro: *Campylobacter concisus* is an emerging enteric pathogen associated with prolonged diarrhea and possibly inflammatory bowel disease in children as well as adults. Previous studies have demonstrated a pathogenic role in epithelial cell functions. The interaction with the immune system is unclear and the magnitude of the systemic immunoglobulin response is unknown. Methods: The opsonic activity in heat-treated (complement depleted) serum from 40 patients with *C concisus* positive stool samples was examined in a chemiluminescence assay reflecting neutrophil oxidative burst response towards a challenge with *C concisus.* The results were compared with opsonic activity in serum from healthy controls. Each serum sample was tested in three separate experiments, all performed in duplicate. Neutrophil cells were isolated from peripheral blood of healthy controls. The opsonic capacity in heat-treated serum is mainly associated with immunoglobulin G activity. Results: A strong activation of the oxidative burst response in neutrophils was demonstrated with *C concisus* opsonised in heat-treated serum. However, the magnitude of peak chemiluminescence did not differ between serum from patients and serum from controls. The opsonic activity of heat treated serum from patients had no association with clinical characteristics (i.e. fever, bloody stools, duration of illness, or age of the patient). Impact of research: From these experiments we observed no indication that the heat-treated (mainly immunoglobulin dependent) serum opsonisation against *C concisus* in patients was higher than normal serum. Probably, the systemic immunoglobulin response towards *C concisus* is weak even in patients with clinical symptoms of prolonged diarrhea.

P64. *Okadaella gastrococcus* found in the stomach of *Helicobacter pylori*-positive young generation

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Background: *Okadaella gastrococcus* (ATCC BAA-2258, NBRC 107862, GenBank HQ699465) is an alpha-haemolytic Gram-negative coccoid facultative anaerobe and is characterized by motility, acid tolerance, and positivity for arginine aminopeptidase and negativity for urease, catalase, oxidase, and PYR tests. They are sensitive to Penicillins, Tetracyclines, Quinolones and Rifamycins but resistant to Metronidazole and Vancomycin. The presence of *O. gastrococcus* in the classic gastric carcinogenic cascades and in *H. pylori*-negative and NSAIDs-negative gastropathies, have been reported. The co-existence of *H. pylori* and *O. gastrococcus* among the young generation is not known. Aim: to examine the presence of *O. gastrococcus* among *H. pylori*-positive young generation. Methods: 20 *H. pylori* positive patients (age: 22–34 years, male: female = 10: 10) were enrolled in this investigation. All patients had gastroesophagoduodenoscopy and biopsy. Targeted biopsy specimens were examined for urease, histology and transmission electron microscopy (TEM). TEM is the one of gold standards to identify *O. gastrococcus*. Results: Intracellular *O. gastrococcus* were found in the stomachs of all patients including in intestinal metaplasia (15%, 3/20). Inflamed gastroesophageal junction and carditis were found in 15 (75%) patients. No Barrett's esophagus was seen. Active chronic gastritis (85%, 17/20), chronic gastritis (15%, 3/20), gastric ulcer (15%, 3/20), gastric erosion (15%, 3/20) and duodenal ulcer (15%, 3/20) were seen. Intracellular *H. pylori* were not seen. Conclusions: *H. pylori* and *O. gastrococcus* co-existed in various gastropathies of all the young patients examined. Further study into the new organism is warranted and an animal model needs to be established for this human pathogen.

P65. Targeting of a homopolymeric G-repeat by a small RNA mediates repression of a chemotaxis receptor in *Helicobacter pylori*

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Bacteria have evolved various mechanisms to adapt to different environmental stresses or hosts. For example, phase-variation represents a frequent and stochastic mechanism of genotype switching that contributes to phenotypic heterogeneity in bacterial populations. Simple sequence repeats (SSR) are the major source of phase variation and have been shown to affect virulence and host adaptation in several bacterial pathogens including *Campylobacter jejuni* and *Helicobacter pylori*. Besides genotypic modifications, bacteria can also rapidly adapt to changing conditions by altering their gene expression. Bacterial small non-coding RNAs (sRNAs) act as post-transcriptional regulators during stress response or virulence regulation. Recently, we discovered an unexpected number of ~60 sRNA candidates based on a differential RNA-seq approach in *H. pylori* 26695. Here we focus on the very abundant HPnc5490 sRNA that represses expression of the chemotaxis receptor *tlpB*, which plays a role in pH-sensing and host colonization. Based on *in-vitro* and *in-vivo* analyses, we could show a direct interaction between the C/U-rich terminator loop of HPnc5490 and a homopolymeric G-repeat within the 5' UTR of the *tlpB* mRNA. Whereas HPnc5490 is highly conserved, the G-repeat within the *tlpB* mRNA shows length variation among diverse *H. pylori* strains. Intriguingly, modification of the G-repeat length in *H. pylori* 26695 affected *tlpB* expression and its down-regulation by HPnc5490. Concordantly, we observed strain-specific HPnc5490-mediated *tlpB* repression in other *Helicobacter* strains with different G-repeat lengths. Overall, HPnc5490 represents the first example of a *trans*-encoded sRNA regulator in *H. pylori* and the first *trans*-acting sRNA that targets a homopolymeric G-repeat.

P66. Antiadhesive activity of *Alpinia katsumadai* seed extract and its post-distillation residue against *Campylobacter jejuni*

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Introduction: Adhesion is a prerequisite step in the infective cycle of pathogenic *Campylobacter jejuni* and a necessary step in disease production. Stopping the adhesion could prevent disease development. For that reason, the focus of the present study was finding a novel agent of plant origin, which could prevent *in vitro* adherence of food isolate *C. jejuni* K49/4 to cell cultures. Methods: We investigated the antiadhesive properties of the ethanol extracts of *Alpinia katsumadai* seeds and the residual material after hydrodistillation of essential oil from it against *C. jejuni* K49/4 in the model of non-polarized pig small-intestinal (PSI) epithelial cells. *A. katsumadai,* a member of the ginger family (*Zingiberaceae*), is widely used in traditional Chinese medicine as an anti-emetic remedy. Both tested extracts were rich in flavonoids and diarylheptanoids, especially alpinetin, pinocembrin and *trans, trans*-1,7-diphenyl-4,6-heptadien-3-one. Cytotoxic and antibacterial activities of the extracts were assessed prior to antiadhesion assay. Results: Seed and post-distillation residue extracts showed significant antiadhesion activity within the larger concentration range from 0,2 μg/ml to 50 μg/ml. At concentrations higher than 0,8 μg/ml, both extracts reduced the adhesion by 2 log units. When concentration of extracts was 0,2 μg/ml the adhesion was reduced by 1 log unit, lower concentrations had no effect compared to control. This effect was more pronounced when the extracts were applied on epithelial cells before infection with bacteria. Impact of research: The results of the present study provide new evidence for beneficial effects of herbal remedies in the treatment of gastroenteritis caused by *Campylobacter jejuni.*

P67. Formate modulates of *Campylobacter jejuni* survival phenotypes

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Formate, a byproduct of fermentation in the hosts' gut, is a primary energy source for *Campylobacter jejuni. C. jejuni* also possesses two terminal oxidases, and formate, in other bacteria, can inhibit these enzymes. Consequently, formate utilization may represent a conflict between energy production and microaerobic survival in this bacterium. To investigate this dichotomy, we assessed here the impact of formate on *C. jejuni*'s survival traits. Chemotaxis assay indicated that wild-type *C. jejuni* 81–176 exhibit enhanced taxis to formate compared to other chemoattractants. Addition of 50 mM formate to growth media also significantly enhanced growth, motility and biofilm formation of 81–176 under microaerobic conditions. However, gradual decrease and a severe deficiency in these phenotypes were observed in media supplemented with higher formate concentrations. Further, tolerance to ambient oxygen was reduced in media supplemented with 50 mM and higher formate concentrations. Interestingly, the oxidase activity was severely reduced in 81–176 when challenged with 50 mM and higher formate concentrations. In contrast, a formate dehydrogenase mutant (Δ*fdhA*), that lacked the ability to metabolize formate, was more sensitive to formate and exhibited defects in growth, motility, and biofilm formation when supplemented with different formate concentrations as compared to wild-type. However, the oxidase activity in the Δ*fdhA* was not as severely affected as in the wild-type. Therefore, we purpose that high concentrations of formate or its catabolic byproducts may inhibit oxidase activity in *C. jejuni*, suggesting that the bacterium may use formate to sense its environment and potentially adapt to prevailing conditions in the gut.

P68. Development of a novel assay for quantification of Helicobacter pylori cell adhesion and findings of anti-adhesive compounds

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Objectives: Studies have shown that different naturally occurring compounds inhibit the binding of Helicobacter pylori to the human gastric mucosa and different types of human cell lines. To enable identification of novel anti-adhesive compounds

and/or plant extracts we designed a quantitative high throughput adhesion assay and screened several compounds. Methods: Four different assays where developed and tested-the most effective one is used here. A 96 well plate were coated with AGS cells and grown to confluent growth ON, wash, for Displacement assay incubation with H. pylori 1h allowing bacteria to adhere, add anti-adhesive extract and incubate 1h, wash three times, cell lysis with 0,05% saponin, add alamarBlue-with the active substance resazurin, which in itself is non-fluorescent but is metabolized to the fluorescent compound resorufin. We show that by adding resazurin to H. pylori adhering to lysed cells, fluorescence intensities can be used to quantify H. pylori adhesion. Three different assays, with varying orders of compound/bacteria adding where used in the screening: Displacement, Competition (mix of bacteria/compounds incubated 1 hour before adding to cells) and Blocking (compounds incubate 1 hour, add bacteria and incubate 1 hour). Results: The anti-adhesive effect on 1 H. pylori strain (SS1) is shown, as there was no difference among tested strains. Part of the results is shown here. Conclusion: We developed a quantitative, high throughput, assay which enable identification of novel anti-adhesive compounds. Three compounds almost completely inhibited the adhesion of H. pylori to AGS cells, regardless of the assay order, Displacement, Competition or Blocking.

P69. Significance of *Campylobacter* IgA and IgG Antibodies in Reactive Arthritis

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Introduction: After *campylobacter* infections reactive arthritis gains increasing importance. Detection of antibodies may characterize post-infectious sequelae. Recombinant immunodominant PEP-antigens, peptidoglycan-associated lipoprotein (OMP18) and ATP/GTP binding protein P39 were described to avoid cross reactivity. Materials: One-hundred patients with enteritis for a mean of 11.3 days (range 1 to 121 days) and *campylobacter* stool cultures were recruited. Serum samples of 84 patients with all study criteria were collected within a mean of 28,7 days (range 8 to 129 days) after receiving stool samples and investigated by commercial *campylobacter* ELISA (Mikrogen Campylobacter ELISA IgG, IgA). A questionaire of participating medical practitioners registered complaints of mono- or oligo-arthritis in patients. Results: 23/84 patients had no *campylobacter* IgG antibodies by *recom*Line assay (Mikrogen). Sixteen patients reported for arthritic joint and/or muscular fatigue symptoms, 13 had positive scores by *recom*Line IgG immunoblot, in *recom*Line IgA only *two* had a positive score. All patients were tested in parallel for antibodies by *recom*Line *yersinia* immunoblot. Except for four patients the majority had positive IgG results, only two a positive IgA result. None of *campylobacter* IgA positive patients were *yersinia* IgA positive. Impact of Research: From the presented data it may be concluded that *campylobacter* IgA antibodies are not found in patients with ReA. Since IgA is considered as one of the primary humoral defense mechanisms at the intestinal mucosa, lack of IgA immune response in infected patients could be one of the requirements for further extraintestinal progression and thus an signal for developing sequelae.

P70. The Role of Putative RNA Degradation Proteins in *C. jejuni* Virulence

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Over the last ten years, there have been nearly 500,000 confirmed cases of *Campylobacter* in the UK, with numbers of cases steadily increasing (HPA). The molecular mechanisms of virulence for *Campylobacter* are still poorly understood, partly due to the lack of tractable virulence models for this organism. In this work, we describe the use of an invertebrate virulence model (*Galleria mellonella*) to identify a highly interconnected and regulated putative RNA degradation system, involved in *C. jejuni* virulence. We have previously defined the roles of PAS-domain signalling proteins in energy taxis. A search of the *C. jejuni* genome revealed the presence of a further PAS-domain linked regulatory protein - Cj1387c. Proteomic analysis of a *cj1387c* mutant showed that the adjacent protein, Cj1388 was over-expressed, suggesting that Cj1387c represses expression of Cj1388. The Cj1388 protein comprises an Endoribonuclease L-PSP domain, which is proposed to inhibit protein synthesis by mRNA cleavage. The *cj1388* mutant is highly attenuated in the *Galleria* virulence model, while the *cj1387c* mutant is moderately attenuated. The *cj1387c* and *cj1388* mutants showed wild-type growth, motility, aerotolerance, and peroxide stress response, suggesting that these phenotypes do not confound the virulence phenotype. Further bioinformatic searching of the *C. jejuni* genome revealed another Endoribonuclease L-PSP domain protein, Cj0327. In the Protein Interaction network, both Cj1388 and Cj0327 are highly connected to proteins that are in turn highly connected in the protein interaction network. In summary, we have identified a novel *C. jejuni* virulence determinant and continue to characterise this system.

P71. The modulatory effects of *H. pylori* infection in the DNA repair mechanisms

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The bacterial pathogen *H. pylori* chronically infects the human gastric mucosa and is the leading risk factor for the development of gastric cancer. The development of gastric cancer is a complex, multistep process involving multiple genetic and epigenetic alterations. Since there are evidences indicating that the bacteria may promote gastric carcinogenesis compromising the integrity and stability of their host's genome, we evaluated the modulatory effects of *H. pylori* infection in the DNA repair mechanisms. AGS cells and the *H. pylori* strain 26695 were employed for co-culture experiments for 4, 12h. To evaluate the effects of infection in the DNA repair mechanisms, it was analyzed the expression of 84 genes using the Human DNA Repair PCR Array (QIAGEN). The methylation pattern of 22 genes was also studied using the Human DNA Repair DNA Methylation PCR Array (QIAGEN). Lastly, the expression of 84 miRNAs was evaluated by means of Human Cancer microRNA PCR Array (QIAGEN). The *in silico* network analysis was performed using MetaCore v6.13 to characterize the biological pathways connecting miRNA-mRNA pairs. Our data indicated that 48% of the genes were significantly down-regulated after *H. pylori* infection. On the other hand, 25% of the miRNAs were up-regulated by the bacteria. Hypermethylation was observed among 14% of the genes. In summary, the in *silico* analysis pointed that *H. pylori* infection could affect the genome integrity impacting the efficiency of the following DNA repair mechanisms: nucleotide excision repair, mismatch repair, non-homologous end joining repair, and homologous recombinational repair.

P72. Gene expression profiling during heat-shock response of *Campylobacter (C.) jejuni*, *C. coli* and *C. lari*

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Although *Campylobacter* species lack typical stress response mechanisms and sigma factors, they are able to survive in the environment and overcome the barriers along the food chain. The response of *C. jejuni* to temperatures above the physiological range is sufficiently characterized. Proteomic analyses and gene expression studies revealed an increased expression of common heat-shock genes and synthesis of corresponding proteins. However survival strategies of *C. coli and C. lari* are still largely unexplored. This study was conducted to examine the response to heat-shock of these two *Campylobacter* species in comparison to *C. jejuni*. First, survival rates at 37 °C, 42 °C, 46 °C and 50 °C were mesured. In our study *C. jejuni* showed a better fitness compared to isolates of *C. coli* and *C. lari*. Gene expression studies by real time qPCR revealed an increased expression of different heat-shock genes (*dna*K, *dna*J, *grp*E, *gro*EL, *gro*ES, *clp*B) in *C. jejuni*, which correlates with earlier studies. The involvement of these chaperones in the heat-shock response of *C. coli* could be demonstrated as well. In contrast, our results for *C. lari* suggest alternative heat-shock response mechanisms, since the analyzed known heat-shock genes did not show an altered expression. RNA-Seq data for *C. jejuni*, *C. coli* and *C. lari* provide an explicit global insight in the diverse transcriptome changes as a consequence of temperature upshift from 37 °C to 46 °C.

P73. Functional analysis of *Helicobacter* polysaccharide lyases

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Aim: Many bacterial organisms produce polysaccharide lyases (PL) in order to facilitate their invasion of host tissues or to use host polysaccharides as a carbon source. *Helicobacter heilmannii* s.l. type-2 are the only epsilon-proteobacterial species possessing putative PL genes. The aim of this study was the functional annotation of *Helicobacter* PLs. Methods: Polysaccharide lyase genes from *H. bizzozeronii* CIII-1^{GEN} (HBZC1_15820) and *H. felis* CS1 (Hfelis_14140) were amplified and cloned in pet28b+. His-tag was fused at the N-terminal terminus of the protein. Both proteins were overexpressed in Rosetta(DE3) pLysS *E. coli* strain and purified using His-Mag Agarose Beads. Capability in degradation of hyaluronic acid (HA), chondroitin sulphate A (CA) and C (CC), dermatan sulphate (DS) and xanthan gum (XG) was evaluated using acid agar method. Major

Findings: Both *Helicobacter* PLs belong to PL family 8, have similar protein domains and do not harbour signal peptide. However, they share only 54% of amino acid identity. Only the recombinant (r-)*H. bizzozeronii* PL showed activity on 3 out of 5 polysaccharides tested, while no lyase activity were observed for r-*H. felis* PL. r-*H. bizzozeronii* PL was able to degrade CA and CC and partially DS. Main conclusion: The *H. bizzozeronii* PL acts as chondroitinase sulphate ABC lyase. Impact of the research: It has been observed that the chief cells of the canine gastric mucosa produce sulphated-moco-saccharide which is depleted on ulcerogenic regimen. The potential role of chondroitinase activity of *H. bizzozeronii* PL in the pathogenesis of gastric disease of dogs is under investigation.

P74. A unique acyltransferase in ε-proteobacteria catalyzes a late step in lipid A biosynthesis

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Escherichia coli synthesizes hexa-acylated lipid A via a nine-step enzymatic pathway conserved in most Gram-negative bacteria. The final two steps involve a set of acyltransferases, LpxL and LpxM, which each add one of the final two acyl chains to complete the lipid A molecule. These last acylation events are critical for the characteristic endotoxic properties of LPS. *Helicobacter pylori*, an ε-proteobacterium, uses this same pathway to synthesize its lipid A, but has only eight homologs to the nine lipid A biosynthetic enzymes, leaving a genetic equivalent for LpxM unknown. Bioinformatic analysis identified *jhp0255* as a candidate gene encoding the "missing" late acyltransferase in *H. pylori*. Attempts to delete *jhp0255* were unsuccessful in *H. pylori* strain J99, suggesting that the gene product may be essential for bacterial viability. However, expression of *jhp0255* was able to complement an *E. coli* strain lacking a functional *lpxM*. As shown by mass spectrometry, Jhp0255 catalyzed the addition of a myristoyl group to the hydroxyl group of the 3′-linked fatty acyl chain of lipid A. This activity was also demonstrated by *in vitro* enzymatic assays using radioactive lipid substrates and purified *E. coli* membranes overexpressing the *H. pylori* protein. Homologs of *jhp0255* were identified in other ε-proteobacteria, including *Campylobacter jejuni* and *Wolinella succinogenes* and corresponding proteins were shown to possess acyltransferase activity. These findings highlight the evolution of a unique set of acyltransferases within ε-proteobacteria with no homology to a functionally identical *E. coli* enzyme.

P75. Effect of capsule on interaction of *Campylobacter jejuni* cells with an analogue of a host cell receptor

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Interaction of *Campylobacter jejuni* with host cells involves a variety of bacterial cell-surface structures. Whilst bacterial adhesins are required for specific binding to host cell receptors, such interaction may be impeded by production of a polysaccharide capsule. In order to elucidate a role of capsule in bacterial interaction with host cells we developed an assay based on bacterial binding to immobilised analogue of a host cell receptor, SBA lectin. The latter specifically interacts with GalNAc residues decorating N-linked glycoproteins located on the cell surface. One example of such glycoproteins, PEB3, was the main focus of this study. This model of attachment was verified in a series of experiments confirming specificity of interaction. Bacterial binding was found to be dose-dependent and affected by the presence of soluble fractions of lectin and GalNAc. Moreover, significant reduction of attachment was observed in several independent *peb3* mutants. In contrast, attachment of non-capsulated *kpsM* was significantly increased. Expression of capsule at certain stages of infection may prevent bacterial recognition by innate host immune system, e.g. by macrophages carrying GalNAc-specific MGL receptors. On the other hand, de-protection of adhesins in non-capsulated cells may be beneficial for bacterial interaction with host tissues at the site of colonisation and attachment. A hypothesis of differential expression of adhesins and capsule was confirmed by qPCR. There was an increase of *peb3* and decrease of *kpsM* gene expression over time. These findings suggest a role of capsule in fine tuning of *C. jejuni* interaction with host cells.

P76. Role of CJSA_0033 in the emergence and pathogenesis of a hypervirulent *Campylobacter jejuni* clone in sheep abortion in the USA

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Introduction: *Campylobacter* is a major cause of sheep abortion worldwide. We recently discovered that a highly virulent, tetracycline-resistant clone of *C. jejuni* (clone SA for sheep abortion) has emerged as the predominant cause of ovine abortion in the USA. This study is aimed to determine the molecular basis of enhanced fitness of this unique clone. Methods: Whole-genome sequence of IA3902 (a clone SA isolate) indicated that it harbored a novel gene (CJSA_0033) that has not been detected in any other *Campylobacter* spp. Distribution of CJSA_0033 in *C. jejuni* isolates from various sources was investigated via PCR. Also, its role in disease pathogenesis was determined using a mouse model of bacteremia and pregnant guinea pig model of sheep abortion. Results: CJSA_0033 has a CG content (22%) considerably less than rest of the genome (30%), and predicted to encode a putative cytoplasmic protein. It contains a region with specific hits to the nucleotide-binding domain of ABC transporters, suggesting it may function in a transporting system. Among the tested *C. jejuni* isolates including those from abortion sources, the gene was found only in clone SA isolates and only after the clone became epidemic. An isogenic *Δ0033* mutant strain was significantly impaired in induction of bacteremia in the mouse and abortion in the guinea pig. Impact of the research: These findings suggest that CJSA_0033 is a horizontally-acquired novel virulence determinant and is involved in the emergence and predominance of sheep abortions caused by a distinct *C. jejuni* clone in the USA.

P77. Investigating the effect of DNA supercoiling on the ability of *Campylobacter jejuni* to associate with a human intestinal cell line.

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Introduction: The structural landscape of DNA has been shown in many bacteria to regulate gene expression on both a local and global scale. DNA supercoiling has been shown to regulate key factors responsible for invasion in *Salmonella* Typhimurium and *Escherichia coli*. Here we describe a clear influence of DNA supercoiling on the ability of *C. jejuni* to interact with epithelial cells. Methods: Adherence and invasion by strains was measured by gentamycin protection assays and immunofluorescence microscopy. DNA relaxation was carried out by the addition of the DNA gyrase inhibitor novobiocin. Cloning into plasmid PMW10 containing a promoterless lacZ gene allowed transcriptional activities of promoter regions to be assessed. Results: Strains with a more relaxed DNA topology were shown to have an increased ability to invade the human intestinal HCT-8 cell line. Relaxation of DNA in more negatively supercoiled strains led to a large and dose dependent increase in invasion of these cells. Transcriptional activities for a range of promoter regions involved in DNA supercoiling and host cell invasion were defined under conditions of DNA relaxation. Furthermore, transformation of these constructs to other *C. jejuni* strains with contrasting DNA supercoiling profiles was used to investigate whether variations in DNA topology between strains results in altered transcription. Impact: This research reveals a role for DNA topology in the regulation of invasion of epithelial cells *in vitro* by *Campylobacter jejuni*. Furthering our understanding of the regulation of this phenotype will be key in order to understand how this pathogen causes disease in humans.

P78. Novel *Helicobacter* Species Isolated from Asian Mice Induce Typhlocolitis in C57BL/6 IL10$^{-/-}$ Mice

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Helicobacter species infections have been reported worldwide in numerous colonies of laboratory mice. Select enterohepatic *Helicobacter* species (EHS) cause inflammatory bowel disease (IBD), colon cancer, hepatitis and hepatocellular carcinoma in mice. These findings have prompted screening for EHS in mouse health monitoring protocols. A novel EHS was isolated from the stomach and intestines of clinically normal mice received from three Asian institutes. The novel EHS was microaerobic, grew at 37°C and 42°C, was catalase and oxidase positive, but urease negative. It is most closely related to the16S rRNA gene of *H. muridarum* (98%); and to the *rpo*B gene of *H. hepaticus* (90%). The novel EHS has *in vitro* CDT

activity; its *cdt*B gene sequence has 83% identity with that of *H. hepaticus*. When the organism was inoculated into C57BL/6 IL10 $^{-/-}$ mice, it was cultured from the stomach, colon and cecum of infected mice at 6 and 10 weeks post infection. The cecum had the highest colonization levels by quantitative PCR. The histopathology of the lower bowel was characterized by moderate to severe inflammation, with associated mild edema, epithelial defects and mild to moderate hyperplasia and dysplasia. Inflammatory cytokines IFNγ, TNFα, IL17 and iNOS were significantly up-regulated in the cecal tissue of infected mice. These results demonstrate novel EHS can induce IBD in IL10 -/- mice and highlight the importance of identifying *Helicobacter spp*. especially when they are introduced from outside colonies from different geographic locations. Exclusion of EHS from mouse colonies will reduce the risk of compromising research results.

P79. DNA supercoiling acts as a key regulator of motility in *Campylobacter jejuni*

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Aim: Changes in the supercoiling of genomic DNA have been found to have a profound effect on virulence gene expression in a number of bacteria. The aim of this study was to investigate the role of DNA supercoiling in the regulation of motility in *C. jejuni*. Methods: Supercoiling levels were manipulated by using sub inhibitory levels of novobiocin and assayed by chloroquine gel analysis. Motility was measured in MH motility plates. The expression of topoisomerase and flagella genes/proteins were monitored by RT-PCR and Western blotting. Major findings: Supercoiling profiles were generated for *Campylobacter* isolates and compared to their motility. A robust correlation between DNA supercoiling and motility was demonstrated. Further confirmation of this relationship was achieved using novobiocin to manipulate supercoiling. Negatively supercoiled isolates were highly motile but became less motile in a dose-dependent manner as the DNA supercoiling levels became more relaxed. FlgRS and flagellin proteins were shown to be regulated through DNA supercoiling. Furthermore, environmental factors had a strong influence on the expression of these proteins. Main Conclusions: 1. DNA topology affects motility, 2. DNA relaxation alters the expression of flagella genes and proteins, 3. FlgR and FlgS have an inverse relationship upon DNA relaxation, 4. Mucin and other environments alter DNA supercoiling and motility. Impact of research: Relatively little is know about the global regulation of virulence genes in *C. jejuni*, this work shows that DNA supercoiling plays a major role in virulence gene regulation and specifically in the regulation of motility through the FlgRS (TCRS).

P80. Detection of Type Six Secretion System (T6SS) in *Campylobacter jejuni* from different sources by PCR assay

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Objective of Study: *Campylobacter jejuni* is one of the common causative agent of gastroenteritis worldwide. However, fewer data is available regarding infections of *C. jejuni* from Asia particularly South Asia. Type Six Secretion System (T6SS) was recently identified in *C. jejuni* that augments virulence and competing advantage over other microorganisms. The aim of the study was to detect the presence of conserved T6SS genes i.e. *VgrG*, *IcmF* and particularly *Hcp* in *C. jejuni* strains from human diarrheal, poultry, livestock and environmental samples collected from UK and Pakistan. Methodology: T6SS gene cluster was recently identified in *C. jejuni* clinical isolate from *Thailand*. For developing a screening PCR assay to test for the presence of conserved T6SS markers, primers were designed to amplify T6SS component conserved genes *IcmF*, *VgrG* and *Hcp*. Screening PCR assay was performed on 112 diverse samples from UK and Pakistan. Results: 13 out of a total of 112 strains were positive for the presence of T6SS marker genes. Positive T6SS strains included five Pakistani strains (one poultry, one waste water and three clinical strains) and eight UK strains (two clinical and six pig strains). Conclusion: Data suggested that the presence of T6SS marker genes in *C. jejuni* strains indicated the presence of functional T6SS and ultimately their enhanced virulence and competitive potential over other bacteria. Presence of functional T6SS in poultry and livestock calls for surveillance of *C. jejuni* in these sources as poultry and livestock are major vehicles in the transmission of *C. jejuni* to humans.

P81. Identification through genome mining of secreted Campylobacter proteins that may be involved in immune avoidance in chickens.

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Aims: To elucidate the cohort of proteins those are predicted to be secreted from Campylobacter jejuni. Of special interest are proteins with no known homologues outside Campylobacter, which therefore may be unique to the organism. These may be involved in crucial species-specific functions. Methods: Standard Bioinformatics tools were used, including SignalP, SecretomeP and BLASTP. Genes encoding identified proteins were amplified and expressed as recombinant proteins, which were subsequently tested for their ability to kill chicken immune cells. Major findings: Of the approximately 1600 proteins encoded by the genome, up to 400 are predicted to be secreted from the bacterium, or inserted into the outer membrane, as identified by using bioinformatics. This includes proteins predicted to contain a signal peptide, and a subset of proteins predicted to be non-classically secreted. Genes encoding several potentially secreted proteins were cloned and recombinant proteins expressed, with one protein exhibiting killing of a chicken immune cell line. Main Conclusion and Impact of the research: Campylobacter is a commensal organism in chickens and therefore either avoids or subverts the chicken immune response. Elucidating how this happens will open up approaches for the control of colonisation in chickens. The approach taken here is to identify the cohort of proteins that may interact with host cells, and test these proteins for activity. The observation of a protein that kills immune cells is highly relevant in the search for the mechanism of immune avoidance.

P82. FIDO-family proteins of *Campylobacter fetus* subspecies *venerealis* represent a functional toxin-antitoxin system

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C. fetus subsp. *fetus* (*Cff*) and *C. fetus* subsp. *venerealis* (*Cfv*) are considered emerging pathogens. *Cff* colonizes the intestinal tract of humans, ruminants, and reptiles and leads to severe systemic disease in immuno-compromised patients. In contrast, *Cfv* is restricted to the genital tract of cattle causing epidemic venereal disease. Despite the distinct niche preferences, comparative genome analysis revealed only minor subspecies-specific variations. One subspecies-specific genome region of *Cfv* carries a pathogenicity island encoding a functional Type IV Secretion System (T4SS) and putative secretion system effector proteins Fic1 and Fic2 containing FIDO-motifs (HPFX[D/E]GNGR). Two additional Fic proteins (Fic3 and Fic4) were identified on the *Cfv*-specific ICE (integrative conjugative element). FIDO-motifs are conserved in bacterial toxin-antitoxin (TA) systems and effectors transmitted to eukaryotic cells during infection. We demonstrate that the toxic FIDO-motifs of Fic2 and Fic3, and the antitoxic inhibitory motifs of Fic1 and Fic4 comprise the first TA systems identified in *Cfv*. Expression of *Cfv fic* genes in *E. coli* led to filamentation and lethality. To explore the putative effector function of these proteins in infection, HeLa cells were transfected with *gfp-fic* fusions. Apoptotic cell death was induced with each putative effector. Nevertheless, T4SS dependent translocation of Fic proteins with a *cyaA*-reporter to host cells could not be verified under laboratory conditions. We propose that the *Cfv* TA systems stabilize horizontally acquired DNA. Additionally, the T4SS Vir-Fic locus may be in a state of evolutionary transition where toxic gene products are acquiring functions of secreted effectors, which will ultimately promote virulence.

P83. The differentiation of *Campylobacter* and *Arcobacter* virulence using adhesion and invasion assays with quantification of cytokines and chemokines and whole genome sequence analysis.

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A selection of *Campylobacter* strains from different sources (chicken, bovine, clinical and water) and *Arcobacter* that had been isolated from market vegetables were subject to adhesion and invasion assays using differentiated caco-2 cells. A sub-set of strains were analysed to determine the immune response of caco-2 cells by the quantification of a range of cytokines and chemokines. To assess which parameter was more indicative of virulence, the ability of different strains to adhere and invade

caco-2 cells was compared to (1) the concentration of cytokines/chemokine's and (2) the range of cytokines/chemokine's detected. Whole genome sequences were analysed for the presence of putative virulence genes. Those identified were assigned an allele number using sequence downloads from the PubMLST *Campylobacter* website. The strains were assigned an ID dependent on the array allele numbers and further analysed to determine if the ID assigned was related to immune response or adhesion invasion parameters. In addition, it was determined if small sub-set of virulence related genes could be used to discriminate between highly virulent strains and those with a lower risk of causing severe symptoms in humans. Further work will be carried out using a larger strain set to predict if the genes identified are able to predict strain infectivity and if any of the above parameters (adhesion and invasion, immune response and virulence genes) are related to source (i.e. clinical, chicken, bovine) or sequence type.

P84. Novel EGFR phosphorylation pathway in H. pylori-infected Gastric Epithelial Cells

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Aims: *H.pylori* activates EGFR signaling by heparin-binding (HB)-EGF via transactivation pathway. In this study, we demonstrated a novel activation pathway of EGFR on gastric epithelial cells via HB-EGF-independent manner. Methods: Human gastric epithelial AGS or MKN-45 cells were co-cultured with *H.pylori*. The activation of EGFR, p65 subunit, p38, ERK, and TAK1 were examined by western blotting. Infected cells were pretreated with or without chemical inhibitors; 5Z-7-oxozeaenol, gefitinib, SB203580, U0126 and the effects on the EGFR phosphorylation of tyrosine, serine, and threonine residues were examined. Flow cytometry was performed to detect the internalization of EGFR. Major Findings: The cells incubated with wild type *H. pylori* were induced the rapid phosphorylation of tyrosine, threonine and serine residues of EGFR with p-p65, p-ERK, p-38 and p-TAK1. 5Z-7-oxozeaenol inhibited the activation of both p38 and ERK with phosphorylation of serine and threonine residues of EGFR. Treatment with gefitinib, SB203580 and U0126 inhibited the phosphorylation of EGFR tyrosine residues, p38 and ERK, respectively. All phosphorylation was abolished in cells with type IV machinery-deleted *H. pylori*. FACS analysis revealed EGFR was internalized after EGFR phosphorylation. Pre-incubation with gefitinib did not block the internalization of EGFR, while SB203580 completely inhibited. Anti-HB-EGF antibody totally inhibited the internalization of EGFR caused by HB-EGF. Conclusion: *H. pylori* induced phosphorylation of serine and threonine residues of EGFR on gastric epithelial cells leading to internalization via activation of p38 and ERK, which is a novel EGFR phosphorylation pathway independent from HB-EGF signaling.

P85. Impact of acquired immunity and dose-dependent probability of illness on Quantitative Microbial Risk Assessment

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Microbial risk assessment comprises several steps. Exposure assessment aims to estimate the exposure of a human population to e.g. *Campylobacter* by food. Dose-response modelling aims to quantify the probability of infection and illness as a function of the ingested dose. Risk characterisation combines the results of exposure assessment and dose-response modelling to arrive at an estimate of the risk of illness at the individual or population level. The standard approach to dose-response modelling is to consider the process ultimately leading to illness as a sequence of events: exposure → infection → illness. Most models and data are available for the exposure → infection probability, there are few models for the infection → illness probability and models with constant probability are suggested as a default. Furthermore, it is typically assumed that the outcomes of subsequent exposures are statistically independent. Our work on modelling the effects of acquired immunity on the dynamics of enteric infections has suggested that this approach overestimates the risk of illness. We present a novel approach to risk characterisation, taking into account both the impact of acquired immunity and dose-dependence of the conditional probability of illness given infection and illustrate by example of risk estimates for *Campylobacter* spp. Recalculating the expected incidence of campylobacteriosis in the Netherlands we obtain results that are more similar to epidemiological estimates. The impact of acquired immunity appears to limit the disease incidence at higher exposure frequencies, whereas dose-dependency of the probability of illness given infection has a major impact at low doses.

P86. Combining phenotypic and proteomic analyses to identify membrane proteins involved in the adhesion of *Campylobacter jejuni* to abiotic surfaces

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As a foodborne microaerophilic micro-organism, *Campylobacter jejuni* can survive transitionally throughout the food processing chain, defying the lethal ambient air. Cross and re-contamination of food products were also reported for this pathogen. Adhesion to inert surfaces could be one of the adaptation strategies developed by *Campylobacter* to survive in the food-processing environment. Using the Biofilm Ring test, we have demonstrated a high intra and inter-species variability in the adhesion capability to inert surfaces among 46 strains of *Campylobacter*. Adhesion capability was also found to be enhanced by environmental conditions and especially in oxidative conditions. A proteomic analysis on each of the two membranes of *C. jejuni* 81–176 indicated that some proteins well-characterized to be involved in motility, adhesion and virulence are over-expressed in oxygen-enriched conditions as compared to microaerobic conditions. Moreover, the transcript level of the gene encoding the virulence factor CadF is enhanced in oxygen-enriched conditions and the adhesion to inert surface of a mutant strain on *CadF* gene was partly impaired in these conditions. Taken together, these data indicate that *Campylobacter* is able to modify its biological and physiological characteristics in conditions out of its natural habitat which favours its survival in food environment sensu lato and enhance its virulence factors.

P87. Identification of Type VI secretion system (T6SS) in Spanish *Campylobacter jejuni*

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Campylobacteriosis is considered a public health concern in the European Union as the reported incidence continues to increase. Recently, a Type VI secretion system (T6SS) was identified in some *C. jejuni* strains that conferred enhanced virulence in cell-based assays and in mice. The gene *hcp* (hemolysin co-regulated protein) was identified as a conserved genetic marker to identify this T6SS. In parallel, *C. jejuni* possesses a variety of genes encoding important oxidative stress response proteins such as superoxide dismutase (SodB), catalase (KatA), or regulators like peroxide stress (PerR) and the newly identified Cj1556. These genes appear to play a role in *C. jejuni* persistence in the environment. In this study, strains from Spanish poultry at slaughterhouse (n=58) and water samples (urban effluent, n=4) were investigated. We performed PCR screening for presence of T6SS and notable genes involved in the production of proteins that play a role in oxidative stress. These, along with the oxidative stress assays, were assessed in order to characterize the *C. jejuni* collection. We found that the majority of strains (79%) possess the T6SS *hcp* gene and display a high level of resistance to oxidative stress irrespective of the isolates' origin. The high incidence of T6SS may be significant *C. jejuni* -associated disease in the EU.

P88. Increase Prevalence of Clarithromycin and Fluoroquinolones resistance among Malaysian *H. pylori* strains

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Backgound: Over the years, eradication of *Helicobacter pylori* has been made difficult due to raising resistance towards multiple antibiotics. Although previous studies been conducted, constant monitoring of antibiotic susceptibility is still essential. Aims: To conduct a comprehensive surveillance study on *H. pylori* susceptibility towards a broad range of antibiotics together with molecular detection to provide a timely insight of *H. pylori* resistance prevalence in Malaysia. Methods: From July 2011 till December 2012, 121 strains were isolated from patients attending the Endoscopy Unit at University of Malaya Medical Centre (UMMC). Antibiotic susceptibility towards metronidazole, clarithromycin, fluoroquinolones (ciprofloxacin, moxifloxacin, levofloxacin, and gemifloxacin), amoxicillin, rifampicin, tetracycline, tigecycline, and nitrofurantoin were

assesed using Etest® strips. Isolated resistance strains were compared at molecular level in *gyrA, gyrB, rdxA, frxA,* and *23S rRNA*. Major Findings: High resistance against metronidazole (33.9%), clarithromycin (10.7%) and fluoroquinolones (6.6%) were reported. Around 8% of these strains were dual resistant. At molecular level, gene mutation at A2146G and A2147G at gene *23S rRNA* are present in all clarithromycin resistant strains. Nonsense mutation in *frx* and *rdx* genes were found in metronidazole resistant strain. Fluoroquinolones resistance is found associated with amino acid substitution in gene *gyrA* at position Asn-87 and Asp-91 and also *gyrB* at position Asp-481 and Arg-484. Main Conclusion: The emerging of clarithromycin and fluoroquinolone resistance among *H. pylori* strains, as well as multidrug resistant strains, are consistent with the overall global trend and important concern in the management of *H. pylori* infections in Malaysia.

P89. Molecular characterization of the Pathogenicity Island in Campylobacter fetus subspecies

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Introduction: The pathogen *Campylobacter fetus* contains two subspecies: subspecies *fetus* (Cff) and subspecies *venerealis* (Cfv). Cfv includes a variant, Cfv biovar intermedius (Cfvi). A pathogenicity island (PAI) of 45kb has been identified with an initially described prevalence of 76% in Cfv and complete absence in Cff strains. The aim of this study was to determine the composition of the PAI in *C. fetus* strains and to investigate the prevalence of PAI-genes in *C. fetus* subspecies. Methods and Results: With BLAST analysis, the PAI-gene sequences of 22 *C. fetus* strains were obtained from Roche 454 shotgun sequencing data. For six strains, the exact gene composition of the PAI was determined using Roche 454 paired-end sequencing data. All PAIs were located on the same position in the core genome, showing that the integration site is conserved. The composition of the PAIs showed an extensive diversity. PAIs are distinguished by insertions and deletions of genes, but the order of genes was almost identical in all PAIs. The prevalence of the PAI-genes was surveyed in a set of 117 *C. fetus* strains of both subspecies from different continents. For this survey, eleven PAI genes on three regions of the PAI were detected by PCR assays. Conclusion: The prevalence of the PAI-genes of 79 Cfvi strains showed extensive diversity. 18 of 29 tested Cfv strains were lacking PAI-genes and 2 of 9 Cff strains harbored PAI-genes, showing that this PAI is not *C. fetus* subspecies *venerealis* specific, but distributed among the subspecies worldwide.

P90. The *Campylobacter jejuni* RacRS two-component system regulates fumarate catabolism and respiration in response to the electron acceptor nitrate

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The natural environment of the human pathogen *Campylobacter jejuni* is the gastrointestinal tract of warm-blooded animals. In the gut, the availability of oxygen is limited therefore less potent acceptors such as nitrate or fumarate are used by *C. jejuni* instead of oxygen. *C. jejuni* has a highly branched respiratory chain and gene expression profiling indicates that a large number of *C. jejuni* genes are expressed differentially in vivo compared to in vitro growth conditions. The regulation mechanisms behind these alterations are however unclear as no homologues of transcription factors known to regulate the energy metabolism in other bacteria exist in *C. jejuni*. Here we demonstrate by transcription analysis and enzyme assays that the response regulator RacR regulates a number of genes involved in energy generation. The activity of RacR is regulated by the cognate sensor histidine kinase RacS as demonstrated by phosphorylation studies and is dependent on the availability of electron acceptors. Footprinting assays showed that RacR binds directly to five promoter elements located upstream of genes directly involved in synthesis of and use as electron acceptor of fumarate. Our results indicate that the failure of the *C. jejuni racR*::cm mutant to colonize chickens may be due to the inability of the *C. jejuni racR*::cm mutant to down-regulate the use of fumarate as electron acceptor when to the more favorable electron acceptor nitrate is present.

P91. Characterisation of the *Campylobacter jejuni* transcriptome using complementary RNA-seq technologies

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Understanding *Campylobacter jejuni* biology is paramount to reducing the amount of *C. jejuni* in the food chain, however our understanding of *C. jejuni*'s transcript structure remains incomplete. The advent of next generation sequencing technologies has afforded new opportunities for both profiling genome-wide transcriptional responses to various stimuli and allowing the identification of genome-wide transcript structures. We have characterised *C. jejuni*'s transcriptome using Illumina HiSeq sequencing and differential 454 pyrosequencing. Illumina HiSeq was used to profile *C. jejuni*'s transcriptional response to iron and inactivation of a Δ*fur*Δ*perR* mutant, whilst differential pyrosequencing identified C. jejuni primary transcripts under standard growth conditions in wild-type and Δ*fur*Δ*perR* strains. The use of two complementary RNA-seq strategies has enabled high resolution assessment of the *C. jejuni* transcriptome, including identification of *C. jejuni* genes transcriptional start sites, mapping of transcript boundaries and operonic structures. These results have also further refined our knowledge of *C. jejuni*'s transcriptional response to iron limitation. In addition, careful analysis of our combined RNA-seq results identified multiple novel transcripts, including several putative intergenic sRNAs. Whilst many of these novel transcripts were identified by both sequencing technologies, several were uniquely identified by either Illumina HiSeq or differential pyrosequencing highlighting the utility of our cross-platform sequencing approach to robustly characterize the contents of the *C. jejuni* transcriptome. The expression of selected novel transcripts was confirmed with RT-PCR and subsequent qRT-PCR results indicate that the expression levels of several of these transcripts are either Fur and/or iron responsive.

P92. Natural transformation of *Campylobacter jejuni* is associated with the flagella

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Campylobacter jejuni is naturally competent for uptake of DNA from the environment, and we previous showed the efficiency of natural transformation to correlate with growth, but nevertheless *C. jejuni* was able to take up DNA even under conditions not supportive of proliferation. DNA uptake systems of Gram-negative bacteria are generally associated with type IV pilus or type II secretion systems, but alternative mechanisms for DNA uptake have been speculated for *C. jejuni*. Investigating natural transformation of *C. jejuni* under acidic and alkaline conditions revealed the pH range of competence to correlate with motility rather than survival and led us to investigate an association between competence and the flagella. We found that Δ*motA* and Δ*flgP* mutants, carrying intact but paralyzed non-motile flagella, showed moderately reduced competence, hence indicating that motility *per se* is not required for natural transformation of *C. jejuni*. In contrast, a *ΔflaAB* mutant lacking the flagella filament displayed significantly reduced competence, showing that the flagella structure is important for natural transformation of this organism. This finding was confirmed by confocal microscopy, which showed fluorescently labelled extracellular chromosomal DNA to bind to the poles, i.e. the site of the polar flagella, of wt cells, while the DNA binding to the Δ*flaAB* mutant was significantly reduced. These findings display a novel association between flagella and natural transformation in bacteria, and we speculate that the DNA uptake machinery is associated with the flagella by employment of the flagella type III secretion system for secretion of competence proteins.

P93. Whole Genome Mapping ™ as a useful tool to determine phylogenetic relationships in *Campylobacter fetus*.

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Campylobacter fetus consists of two subspecies, *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*, that are phenotypically very distinct, but are hard to separate by genotype. *Campylobacter* species are known to show a high degree of horizontal gene transfer and recombination events, making it difficult to determine phylogenetic relationships reliably. Whole Genome Maps™ (Opgen Inc.) are genome-wide, ordered restriction maps from single molecules of DNA. They serve as a unique barcode for an isolate and can be used to track horizontal gene transfer and recombination events. Whole genome maps (WGM) from both subspecies of *C. fetus* were made and whole genome sequences (WGS) were obtained using Roche 454 and Illumina MiSeq sequencing. Phylogenetic clustering of the strains was performed in BioNumerics based on the WGM and the WGS data and the results of both techniques were compared. Clustering based on the WGM provided a good resolution between the two subspecies of *C. fetus*. The detection of recombination and insertion events was easier and quicker using WGM compared to WGS data, especially when the WGS data has insufficient coverage to obtain a reliable assembly. However, these events should be interpreted with caution and preferably be confirmed by a more detailed analysis of the sequence at these positions.

P94. Subtyping of *Campylobacter* species with BioNumerics

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Campylobacter species are one of the most common causes of diarrheal illness in the developed world. In persons with compromised immune systems, it may be life threatening. Many countries have a national surveillance system for campylobacteriosis. However, subtyping of strains is not always performed and furthermore, typing methods differ between countries, making it difficult to detect outbreaks across national borders. Until today, PFGE is considered the "gold standard" in most surveillance networks. However, because PFGE is time-consuming, labor-intensive and its interpretation prone to errors, we will demonstrate how whole-genome sequence (WGS) based methods can be implemented in routine molecular surveillance. The BioNumerics software suite acts here as a backbone for data preprocessing, submission, curation, identification and cluster detection. Here, we show that PFGE clusters can easily be detected by WGS-derived data, such as gene-by-gene systems and K-mer SNPs, both reference-based as de novo. The polyphasic setup of the BioNumerics environment allows validation of the WGS results with "traditional" approaches. This encompasses not only the validation using phenotypic data (resistance, virulence, . . .), the molecular typing techniques (PFGE, MLVA, . . .), but also includes the integration of WGS data through an in silico approach with existing data. Rapid and automatic processing by push-button applications is needed to ensure a reliable and easy to follow workflow in routine molecular surveillance, reducing the time needed to detect and contain potential outbreaks, eventually reducing the cost on public health and food safety.

P95. Shotgun proteomic analysis of *Campylobacter fetus* subsp. *fetus*

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Campylobacter fetus subsp. *fetus* is a major cause of sheep and cattle abortion in the UK and worldwide, as well as causing opportunistic infections in humans, including bacteremia, meningitis and other systemic diseases in immunocompromised individuals. The surface of *C. fetus* is covered by a high molecular weight proteinaceous surface layer (S-layer) which is a major virulence factor, having roles in adherence, immune evasion and antimicrobial resistance. Additional bacterial factors with roles in disease are yet to be characterised. In this study, a rapid shotgun proteomics-based approach was used to

catalogue the main protein components of the ovine abortion-associated strain *C. fetus* subsp. *fetus* 03509. In order to maximise protein detection, triplicate samples were fractionated by differential detergent solubilisation. Protein fractions were analysed using SDS-PAGE followed by one-dimensional monolithic column liquid chromatography- electrospray ionisation (ESI) and fast MS/MS scanning. This approach enables the analysis of complex protein mixtures, including hydrophobic proteins, while simultaneously combining rapidity with breadth of coverage. MS/MS data were searched against the cognate database, generated following 454 sequencing of the genome of strain 03509. Following confident identification of more than 500 proteins, *in silico* functional analysis was carried out to predict protein localisation and assign putative functions with particular attention on environmental adaptation, and virulence.

P96. Impact of broiler type on inflammatory responses to *Campylobacter* infection: considerations for gut health and bird welfare

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Standard production systems (STD) constitute ~90% of UK broiler production and use birds reaching slaughter weight at 35–39 days (fast-growing; FG). Higher welfare (HW) systems account for the remaining 10% and typically use slower-growing (SG) breeds, reaching slaughter weight at ≥48 days. Studies suggest that HW flocks have lower *Campylobacter* levels and reduced incidence of welfare-associated pathologies than STD flocks. Here, we tested the hypothesis that broiler type, which encompasses growth rate under controlled conditions, is important in determining the response to *Campylobacter*. 10^5 cells of *C. jejuni* M1 were administered orally to age-matched chicks (n=200) from two commercial broiler breeds, 'FG' and 'SG', at 21 days old. Birds (n=60) were killed at 2, 5 and 12 days post-infection (dpi), and caecal contents collected for bacteriological quantification. RNA extracted from caecal tissue was used for qRT-PCR to examine local cytokine responses in the intestine. Caecal colonisation levels were similar in both lines by 2dpi. At 12dpi however, caecal *Campylobacter* load was higher in FG birds than in SG ones, but this was not statistically significant. FG birds also exhibited higher levels of pododermatitis than SG ones at 12dpi. In the caeca, FG birds displayed prolonged expression of pro-inflammatory cytokines CXCLi2 and IL-1β compared to SG ones, and displayed lower IL-10 and IFNγ expression at 12dpi compared to SG birds. These data suggest that the response of SG birds may more tightly regulate gut inflammation than that in the FG breed, contributing to better gut health in such birds.

P97. Comparative analysis of three cytolethal distending toxin genes in *Campylobacter hyointestinalis* and its gene products

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Cytolethal distending toxin (CDT) is one of the most well characterized virulence factors in *Campylobacter* spp., which causes progressive distension and cell death against susceptible eukaryotic cells. We have previously reported the *cdt* genes in *Campylobacter hyointestinalis* isolated from Thai patient with diarrhea. In the present study, we have detected two novel *cdt*-gene variants (*chcdt-II*, and *-III, chcdt-I* for prototype) in *C. hyointestinalis*, and investigated genetic diversity, distribution and biological activities of ChCDTs comprehensively. Nucleotide sequences of novel *cdt* genes were determined by genome walking. The distribution of each *cdt* genes was examined in 24 *C. hyointestinalis* strains by colony hybridization. Representative *cdtABC* genes were cloned, their gene products were expressed in *E. coli* strain and characterized in various aspects. Nucleotide sequences analysis revealed that *cdt-II* and *cdt-III* genes also encoded three adjacent genes, designated as *cdtA*, *cdtB*, and *cdtC*. The homologies of the deduced amino acid seuqneces between ChCDT-I, ChCDT-II and ChCDT-III were: CdtA, 25.3–48.2%; CdtB, 54.7–75.2%; CdtC, 25.1–52.8%, respectively. Colony hybridization revealed that the *chcdt-II* genes were detected in all 24 strains tested whereas the *chcdt-I* and *chcdt-III* genes were detected in 88% and 21% strains, respectively. Recombinant ChCDT-II and ChCDT-III also caused cell distension and cell death against certain cell lines but ChCDT-II had no effect on CHO cells. These data suggest that *C. hyointestinalis* produce more than one CDT (up to 3 CDT) and ChCDTs may be a possible virulence factor of *C. hyointestinalis* but function of each ChCDT in the pathogenesis remains unclear.

P98. Impact of biofilm formation by *Helicobacter pylori* on antibiotics susceptibility

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The human gastric pathogen *Helicobacter pylori* forms biofilms *in vitro* and *in vivo*. Biofilm bacteria could express properties distinct from planktonic cells, one of which is an increased resistance to antimicrobial agents within the same microorganism. The aim of this study was to evaluate the effect of biofilm formation by *H. pylori in vitro* on drug susceptibility to clarithromycin, metronidazole, and amoxicillin, which are used for first- or second eradication therapy of this microorganism. First, we determined clarithromycin susceptibility using growing *H. pylori* biofilm on glass coverslip surface. *H. pylori* biofilm biomass was increased after treatment of clarithromycin. Furthermore, minimum bactericidal concentrations of clarithromycin against *H. pylori* in a biofilm were higher than that against planktonic cells. It was shown that the expressions of RND family of efflux pumps were significantly increased in biofilm cells. Since the participation of the efflux pumps in the development of multidrug resistance has been reported in *H. pylori*, we examined the effects of biofilm formation on the susceptibility to metronidazole and amoxicillin. The biofilm cells were more resistant to these antibiotics than the planktonic cells. Next, we examined the generation of clarithromycin resistance in a spontaneous mutant strain. Exposure of biofilm to clarithromycin showed a high level of resistance generation compared to planktonic cells. These results indicated that *H. pylori* biofilm cells could decrease the susceptibility to antibiotics compared to planktonic cells. In addition, *H. pylori* clarithromycin resistance are more frequently generated in biofilm than in planktonic cells.

P99. Specific and cross reactive antibodies detected from the lipopolysacchride sensitized rabbit

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Aims: New Zealand White rabbit immunized by lipopolysacchride (LPS) from *C. jejuni* is a good model to study the relationship between *C. jejuni* and Guillain–Barre´ syndrome (GBS). The immunization experiment using the purified LPS from different *C. jejuni* strains isolated from GBS outbreak cases, GBS sporadic cases, and diarrhea cases controlled with *E.coli* were investigated in this study. Methods: LPS was extracted by hot phenol-water method and quantified using standard curve method. 41 NZW rabbits (17 groups) were immunized with LPS and whole cell lysate, respectively. Antibodies against LPS, whole cell lysate and the cross reactivities to different gangliosides were tested with ELISA. Major findings: IgG against LPS were found being gradually increased during the immunization period both in the serum of LPS and whole cell sensitized rabbits. The cross-reactive antibodies to GM1a were detected from all of the tested immuno rabbit groups except the group which were injected with *C. jejuni* ICDCCJ07001 and its LPS. The serum immunized with *C. jejuni* 07002 had strong reaction with GT1a. Whole cell lysate could induce higher anti-LPS antibodies than purified LPS. Main Conclusion: LPS from *C. jejuni* ICDCCJ07001 had different ganglioside mimic with the LPS from *C. jejuni* ICDCCJ07002. Impact of research: Animal experiment was not only useful to investigate the immunogenicity and antigenicity of the LPS from *C. jejuni* but also had power to discriminate the structure difference with anti-gangliosides' cross-reactive tests.

P100. *Helicobacter Pylori* Biofilm: Electron Microscopy Analysis

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Introduction: Biofilm formation is critical for successful infection for numerous pathogenic bacteria including *Helicobacter pylori* (*HP*). Little is known regarding the mechanisms of *HP* biofilm formation and the ultrastructure of *HP* biofilm. Methods: Refrozen reference and freshly isolated *HP* cultures as samples of gastric mucosa (GM) under peptic ulcer disease were studied using scanning (SM) and transmission (TM) electron microscopy. "Quanta 200 3D" ("FEI Company", USA) and "JEM-100B" ("Jeol", Japan) systems were used for SM and TM, respectively. Standard dehydration technique was used for TM of ultrathin sections of GM, but not for SM of samples. Glass and steel were used as abiogenic carriers of biofilm producing culture. Results: As shown using SM *HP* formed biofilm with manifest extracellular matrix on the surface of both types' carriers. SM also revealed *HP* cells deeply immersed in the amorphous biofilm matrix covering apical surface of

epithelium layer of GM and including fibrin insertion. As shown using TM *HP* cells were located predominantly on the surface of epithelial cell, which forms outgrowths enveloping bacteria without phagosome formation. Sometimes *HP* cells were obtained in cytoplasmic space of mucus producing cells. A significant number of *HP* cells had defects of cell wall. Protoplasts in submucosal layer were occasionally detected also. Large pores were formed in biofilm matrix of old *HP* cultures. Impact Of Research: *HP* has the ability to biofilm formation both *in vitro* and in natural conditions. Understanding of *HP* biofilm development can contribute to progress in the prevention and treatment of gastroduodenal diseases.

P101. Cytokine induction and virulence mechanisms of *Arcobacter butzleri* in human macrophages

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Arcobacter butzleri (A. butzleri) is a member of the family *Campylobacteraceae* and is considered an emerging zoonotic pathogen. Although no obvious effects on animal health has been reported, severe gastroenteritis can occur in humans e.g. via consumption of contaminated food, mainly pork and poultry. However, the molecular mechanisms of the human innate immune response to *A. butzleri* remains poorly understood. To evaluate the influence of *A. butzleri* on human macrophages, three different strains (human as well as chicken isolates) were studied in an *in vitro* infection model using macrophages derived from the monocytic cell line THP-1. Custom RT-qPCR arrays were used to investigate mRNA expression of relevant cytokines typically produced in macrophages during infection. The expression of IL-1a, IL-1b, IL-6, IL-8, IL-12b and TNF was highly induced by all strains while IL-12a and iNOS remained unaffected. Survival assays revealed the ability of the pathogen to survive and resist the hostile phagosomal environment of phagocytic immune cells. Current attempts are directed towards the ability of *A. butzleri* to induce apoptosis in macrophages. In this context, the potential role of IL-1b triggering the Caspase 8 pathway is examined. The current study will help to gain insight into the molecular mechanisms of host-interaction with this emerging zoonotic organism enabling the generation of future strategies targeting *A.butzleri* induced diseases.

Epidemiology and Evolution

P102. *Campylobacter* spp. is it a problem among healthy children in rural Bangladesh?

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To determine the prevalence of *Campylobacter* spp. and their drug resistance from children less than 5 years of age, we carried out a case-control study in a rural district in Bangladesh. During 2008–2010 and 2012, stool specimens were collected from 2,136 children with diarrhea (cases) and from 4,123 age-matched controls from the same source population. Stool samples were inoculated onto Campy Brucella agar plates (Difco, USA) and incubated at 42°C in a microaerophilic condition for 48 hours. Suspected colonies were identified as *Campylobacter* spp. based on colonial and gram stain morphology, positive oxidase, catalase and hippurate hydrolysis test. Overall, *Campylobacter* spp. were isolated from 304/2136 (14.2%) cases and 620/4123(15.04%) controls. In the study period, *C. jejuni* was identified more than other *Campylobacter* spp, 74.56% versus 25.44% in 2008–10 and 77.9 % versus 22.1% in 2012. *Campylobacter* spp. was found significantly more in control than in children with less severe diarrhea (5.1% versus 12.05%, $p<0.0001$). Among control < 12 month of age, *Campylobacter* spp. was found in higher percentage. Coinfection with two or more pathogen was found in more than 81% of the *Campylobacter* positive patients. Multidrug resistance (resistant to three or more antibiotics) was detected in 86.91% and 93.03% isolates in 2008–2010 and 2012 respectively. Resistance to ampicillin increased from 31.03% in 2008–2010 to 50.8%; ($P<0.0001$), and tetracycline from 76.8% to 85.7% in 2012, $P<0.003$. For erythromycin 2.4% in 2008–2010 and 8.6% in 2012, $P<0.0001$. Long term surveillance of asymptomatic children needed for prevention of *Campylobacter* infection.

P103. Opposing and Combinatorial Effects of Bottlenecks and Phase Variation on the Genetic Diversity of *Campylobacter jejuni* Populations

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The genome of *Campylobacter jejuni* contains multiple hypermutable poly-G tracts. Alterations in length of these tracts occur frequently and can cause phase variation in protein expression usually by altering the reading frame and causing truncation of protein coding regions. The life cycle of *C. jejuni* is likely to involve one or more population bottlenecks, each potentially reducing the population to a single individual. One suggestion as to the functional purpose of this abundance of phase variable genes is to rapidly re-establish population diversity after severe population contractions. Here we report on *in vitro* and *in silico* investigations of the effect of bottlenecks on population diversity. Samples of the NCTC11168 strain were serially passaged on plates, or in broth, with varying sizes of bottlenecks artificially imposed between passages. The state of all 29 phase variable regions was then determined by PCR analysis to determine the impact of the bottlenecks on population diversity. Our data show that the size of the bottleneck impacts diversity in the population, with smaller bottlenecks producing populations that are more variable with respect to the starting population. We also identified evidence of a selective effect on phase variable loci when passaging through broth with experimental populations differing in agglutinating phenotype and expression states of some phase variable genes. Finally, we developed an *in silico* simulation of the effect of bottlenecks based on theoretical models of phase variation and bottlenecks and compared the results of this computer based approach to the experimental data.

P104. Is *Campylobacter jejuni* metabolic diversity related to host species?

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Campylobacter jejuni is the most common cause of foodborne disease in both the developed and developing worlds. However, our knowledge of infection mechanisms is significantly limited. Recently, it has been suggested that diversity in three

Campylobacter jejuni metabolism-related genes could have a major influence in colonization and pathogenicity. These include γ-glutamyl-transpeptidase (*ggt*), fucose permease (*fucP*), and secreted L-asparaginase (*ansA*(s)), which enable the metabolism of glutamine, fucose and asparagine respectively. This study analysed the association of 57 previously MLST-typed *C. jejuni* UK isolates from humans, chickens and cattle with the presence of *ggt*, *fucP* and *ansA*(s) genes using PCR. We found that the presence of *fucP* was more common when compared with the presence of both *ggt* and *ansA*(s), which were less frequent within our isolate collection. A strong association between many clonal complexes (CCs) including ST-21 CC, ST-48 CC, ST-257 CC, and the presence of *fucP* was observed. ST-42 CC and ST-45 CC isolates displayed variable combinations of the three metabolic genes. Absence of all three metabolic genes was detected in isolates belonging to the ST-61 CC and the one isolate belonging to the ST-206 CC. Furthermore, strains which are categorized as "Orphan" STs, were also negative for all the metabolic traits. These results revealed that the three metabolic genetic traits *ggt*, *fucP* and *ansA*(s) are related to certain host-associated sequence types and clonal complexes.

P105. Detection of IgA in the Small Intestine of Chickens after Colonization with *Campylobacter jejuni*

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Campylobacteriosis is one of the most reported bacterial food borne gastro-enteric diseases and poses a serious health problem. In industrialized countries handling, preparation and consumption of contaminated chicken meat is considered to be the main source of infection of humans. Our group is involved in the development of vaccines against *C. jejuni* as part of the strategy to reduce the level of colonization of poultry by this zoonotic pathogen. To develop an effective vaccine it is necessary to understand the immune response that occurs during colonization of the gut by these bacteria. An efficient and effective method to collect mucous containing IgA will also be central to the development of a vaccination strategy. With this in mind we orally challenged birds with *C. jejuni* and investigated several methods to collect mucous from their small intestine. We compared the results obtained from infected and uninfected birds. All methods used the 6 inches of small intestine proximal to the cecal arms; the particulate matter was removed by centrifugation. Samples were stored at –20°C until used in ELISA plate assays. We observed that the *C. jejuni* FlaA protein used to coat the plates was superior to the whole bacterial cells as it resulted in a lower background in the negative samples. In all collection methods significant variation was observed in the level of IgA detected between individual birds. Little difference was observed in the level of IgA detected among the techniques used.

P106. Oral colonisation of *Campylobacter concisus* in different age group

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Campylobacter concisus is a fastidious gram-negative, curved-rod bacterium which is normally found in the human oral cavity, but was suggested to be an emerging pathogen due to its isolation from periodontitis and gastroenteritis. In children, *C. concisus* was previously reported to be more frequently isolated from permanent than deciduous teeth within the 7–8 years age group with mixed dentition. In comparison fewer numbers of this bacterium were found in children with deciduous teeth, where the greater number of isolates was present in the molars than incisors. In this study we investigated the presence of *C. concisus* in families with children before teething. Gum swabs were collected from seventeen healthy volunteers and cultured on horse blood agar using the filtration method. Genomic DNA was extracted from typical colonies and the identity of the isolate was confirmed by PCR amplification. A total of twenty-four *C. concisus* oral isolates including five isolates from the non-teething children were obtained. The strains were then grouped into two genomospecies (A&B) according to the PCR amplification of the 23s rDNA. Interestingly, all strains collected from the non-teething children belonged to genomospecies B. Furthermore, the strains isolated from members of the same family were compared using SDS-PAGE in order to identify the similarities within their outer membrane proteins (OMPs) profiles. To our knowledge, this is the first report on isolating *C. concisus* from healthy, non-teething children. Further studies are required to confirm the colonisation patterns of this bacterium in this age group.

P107. Investigations of changes to campylobacter numbers on broiler carcasses during and following processing.

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Aims: To construct microbiological profiles of operations in chicken broiler slaughterhouses in the UK which show how numbers of campylobacters change during the various processing stages. Methods: Neck skin samples were collected immediately after the process stages of exsanguination, scalding, de-feathering, evisceration, cropping, inside-outside washing and initial (non-maturation) chilling. The colonisation statuses of the sampled flocks were confirmed by testing caecal contents for numbers of campylobacters. All testing was using the ISO 10272-2 protocol. Major Findings: In seven of the 23 processing lines profiled, the initial chilling stage of processing caused statistically significant reductions in the numbers of campylobacters isolated from neck skins. In 11 of the lines studied, there were no significant changes to *Campylobacter* numbers between any consecutive processing stages. However, in these lines there was an overall significant reduction to numbers between the post-pluck and the post-chill stages of processing On a single line, the inside outside washing stage of processing caused significant reductions in the numbers of campylobacters. Main conclusion: Poultry processing is highly automated but there are some stages on some process lines which can effectively reduce *Campylobacter* numbers on carcasses. Impact: The identification of stages on some processing lines which remove a significant number of campylobacters from the birds suggests that it may be possible to modify lines such that they reduce the numbers of campylobacters on finished carcasses.

P108. Comparison of the stomach microflora from *Helicobacter pylori* infected patients and uninfected patients

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Introduction: Stomach is regarded as an environment without bacterial colonization, with the exception of *Helicobacter pylori* (Hp) which may cause chronic gastritis, peptic ulcer and gastric adenocarcinoma. The stomach becomes more susceptible to colonization by other organisms when there is a reduction in level of acid induced by Hp. The aim of this study was to compare the stomach microflora in Hp infected patients and uninfected patients. Methods: A total of 45 patients, who underwent upper gastrointestinal endoscopy because of dyspeptic symptoms were selected for Hp detection and bacterial examinations. Biopsies were plated onto blood agar and pylori agar (bioMerieux) and were incubated under microaerophilic condition at 37ºC. All non-Hp bacterial isolates were identified by MALDI-TOF MS (Bruker Daltonics). Colonies were analyzed by direct deposition on the target plate or after formic acid extraction. Epi Info was used for statistical analysis. Results: 10 patients were Hp positive (7 of them had non-Hp bacterias, 70%) and 35 were negative (11 of them had non-Hp bacterias, 31,4%). This difference was statistically significant (p=0,028). Genera identified by MALDI-TOF in Hp+ patients were: 6(23.1%) *Actinomyces,* 1(3.8%) *Capnocytophaga,* 2(7.7%) *Gemella,* 3(11.5%) *Neisseria,* 3(11.5%) *Rothia,* 11(42.3%) *Streptococcus.* Gener identified by MALDI-TOF in Hp- patients: 6(12,2%) *Actinomyces,* 1(2%) *Clostridium,* 1(2%) *Corynebacterium,* 1(2%) *Eikenella,* 4(8,2%) *Gemella,* 2(4,1%) *Moraxella,* 6(12,2%) *Neisseria,* 2(4,1%) *Propionibacterium,* 8(16,3%) *Rothia,* 2(4,1%) *Staphylococcus,* 16(32,6%) *Streptococcus.* Conclusion: In 70% of Hp+ patients, non-Hp bacteria were isolated whilst only 31.4% in uninfected patients. The presence of Hp seems to favors the presence of non-Hp bacteria.

P109. Prevalence of *Campylobacter* spp. in wild caught and stranded neonatal and juvenile grey seals (*Halichoerus grypus*) in Scotland

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Campylobacter spp. have been isolated sporadically from several species of marine mammals worldwide. However, their prevalence, genotypic and phenotypic characteristics and significance remain poorly understood. The relatively recent description of the thermophilic *Campylobacter insulaenigrae*, a close relative of *C. jejuni* and *C. lari*, in some species of marine

mammal and a single human patient with end-stage renal and hepatic disease, has sparked debate as to whether this novel species of *Campylobacter* may be specific to marine mammals. The prevalence of *Campylobacter* spp. was assessed in rectal swabs from 200 grey seals (*Halichoerus grypus*) (122 live pups, 59 dead pups and 19 live juveniles) from Scottish coastal waters and evaluated with regards to life stage, spatio-temporal distribution, breeding colony substrate and pathology. For grey seal pups the prevalence was 42.2% (76/181) and *Campylobacter* were not isolated from rectal swabs from any of the juveniles (0%; 0/19). Extensive phenotyping was performed with *C. jejuni* (n=88) and a non-hippurate hydrolysing thermophilic *Campylobacter* sp. being identified (n=93). Although biochemical and phenotypical characteristics of the second group were most suggestive of *C. insulaenigrae* or *C. lari*, molecular typing using next generation sequencing is currently underway to fully identify these isolates and to examine their relationship with *Campylobacter* spp. found in the terrestrial ecosystem. This is the first report of *Campylobacter* spp. in grey seals and a step towards understanding spread and evolution of this bacterium in the marine environment along with their relationship to isolates originating from livestock, wild birds and domestic sewerage.

P110. Incidence of *Campylobacter* Species in Free Range and Commercial Broilers in South Africa

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Introduction: A brief literature survey reveals that 35% to 85% of poultry flocks worldwide are infected with campylobacters. These figures suggest that broilers are the main source of contamination in the domestic and commercial setting. Consequently, a high risk of contracting campylobacteriosis is associated with the mishandling and consumption of contaminated chicken. The goal of this study was to determinethe incidence rate of *Campylobacter* species in South African free range and commercially-bred broilers. Methods: The ISO 10272-1:2006 protocol and modified Cape Town Protocol was used to isolate, identify and speciate *Campylobacter* from fresh chicken meat. The samples were received directly from several abattoirs representingfree range and commercial farms in the Western Cape and KwaZulu Natal provinces of South Africa. The sampling period of this study took place from April 2011 to August 2012. Results: A total of 156 *Campylobacter* strains were isolated in this study. *C. jejuni* were more frequently isolated (65%, 102/156) than *C. coli* (33%, 51/156). The remaining 2% (3/156) of the isolates could not be clearly discriminated. The present study indicated that South African free range chickens had a higher incidence (65%, 118/182) of *Campylobacter* carriage than commercially-bred chickens (45%, 38/84). Impact of research:The consumption of free range products is becoming a popular trend worldwide for its health benefits. This study confirms that free range chicken is not always the safer option for consumption. Therefore all chicken, free range and commercially-bred, requires proper cooking and handling to reduce the chance of contracting campylobacteriosis.

P111. Identification, strain typing and plasmid profiles of *Campylobacter* isolates in KwaZulu-Natal, South Africa.

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Introduction: The objective was to describe similarities/differences in the plasmid profiles and strain types of *Campylobacter* spp. from different poultry farming systems in KwaZulu-Natal, South Africa. Additionally, it compared phenotypic and genotypic identification methods of *Campylobacter* against the gold standard of polymerase chain reaction (PCR) in terms of sensitivity and specificity. Methods: Thermophilic *Campylobacter* isolates were identified using conventional biochemical tests, MALDI-TOF mass spectrometry and PCR with primers unique to *C. jejuni* and *C. coli.* Plasmid analysis was undertaken using an alkaline lysis method and PFGE was executed according to the Pulse Net protocol. Results: The MALDI-TOF was shown superior to biochemical tests for the identification of *C. coli* but equivalent to the biochemical tests for *C. jejuni.* Plasmids were harboured in 84 % (16/19) of the isolates from the free-range broilers, 83 % (10/12) of isolates from industrial layers, 72 % (18/25) of the isolates from industrial broilers, and 77 % (10/13) of isolates collected from rural or informally reared poultry. The PFGE genotyping of 42 *Campylobacter* isolates generated 39 *Sma*I types (47.6 % in C. coli, 33.3 % in *C. jejuni* and 2.4 % *C. lari*) of which 28 % were amongst industrial broilers, 26 % amongst the free-range broilers, 26 % amongst industrial layers, and 21 % amongst rural or informally reared poultry. Research impact: There were no correlations of any of the parameters within and between farming systems attesting to the diversity and complexity of phenotypes and genotypes indicating *de novo* evolution to antibiotic selection pressure.

P112. Prevalence of Penner serotypes by multiplex PCR of capsular genes among Campylobacter jejuni isolated in South and Southeast Asia

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Introduction: Campylobacter jejuni is one of major bacteria responsible for human gastroenteritis worldwide. Multiplex PCR of different capsular genes are evaluated to detect Penner serotypes among C. jejuni from South and Southeast Asia. Methods: C. jejuni isolated from children with diarrhea in Thailand (n=513), Nepal (n=94), Cambodia (n=24) and Bhutan (n =21) together with isolates from traveler's diarrhea in Nepal (n=46) and Thailand (n=58) were studied. We performed three multiplex PCR sets using 25 different primers recognized 27 Penner's (HS) and 3 complexes (cpx) serotypes. The results obtained from traveler' diarrhea was compared to the isolates from indigenous population. Results: The five most common Penner serotypes (HS) of isolates from travelers' diarrhea were HS3cpx (13.5%), HS2 (10.6%), HS15 (9.6%), HS53 (6.7%) and HS4cpx A+B (5.8%). The five most common Penner serotypes among isolates in indigenous population were HS2 (17.5%), HS8 or HS17 (12.4%), HS3cpx (8.9%), HS4cpx A+B (7.5%), and HS4cpxA (6.7%). The frequency of untypable isolates ranged from 0-5% in Thailand and Bhutan and as high as 13-21% from isolates in Cambodia and Nepal. Among the remaining untypable isolates by multiplex PCR, the three most common Penner serotypes of these isolates were HS11, HS40 and HS57. Impact of research: This study demonstrated heterogeneity of Penner serotypes in travelers and indigenous population by multiplex PCR for capsular genes. The multiplex PCR identified Penner serotypes in 80-100% C. jejuni strains. Addition of primers specific to capsular gene sequences from HS11, HS40 and HS57cpx in multiplex PCR will improve Penner serotype identification.

P113. Isolation and characterization of *Campylobacter jejuni* bacteriophages from free-range poultry farms using different Penner serotypes expressing a variety of surface structures

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In this study we isolated novel bacteriophages infecting the zoonotic bacterium *Campylobacter jejuni* that may be used for phage therapy of colonized poultry to prevent spreading of the bacteria to meat products causing disease in humans. Most *C. jejuni* phages have been isolated using NCTC12662 as indicator strain, which may have biased the selection of phages. A large group of *C. jejuni* phages rely on the highly diverse capsular polysaccharide (CPS) for infection and recent work identified the *O*-methyl phosphoamidate modification (MeO*P*N) of CPS as a phage receptor. We therefore chose *C. jejuni* expressing different CPS structures as indicator strains in a large screening for phages in samples collected from free-range poultry farms in Denmark. Phages were isolated using *C. jejuni* NCTC12658, NCTC12662 and RM1221 as indicator strains and 21 of these phages were further characterized. Three phages with different host range, genome size and DNA restriction profile were isolated from the same farm, but from three different indicator strains, indicating that the indicator strain strongly influence which phages that are selected for. Most phages were isolated using *C. jejuni* NCTC12662 and RM1221 and genome size (145 kb vs. 194 kb), host range, dependency on MeO*P*N and morphological appearance correlated with isolation strain. Currently, we investigate if CPS is required for infection of these two groups of phages.

P114. Chicken juice is a conditioning matrix which allows more efficient attachment of *Campylobacter jejuni* to abiotic surfaces

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The majority of bacteria exist in either single or multi-species biofilms, leading to increased tolerance to starvation, antimicrobials and environmental extremes. One of the conundrums of *Campylobacter jejuni* as foodborne pathogen is its successful survival in the aerobic conditions in the food chain, despite sensitivity to atmospheric oxygen levels. We previously showed that *C. jejuni* biofilm formation is increased in aerobic conditions in standard growth media; here we build on this work by investigating biofilm formation in chicken juice, representing an *in vitro* model for food-chain relevant conditions on abiotic surfaces. As crystal violet staining was not suitable for measuring biofilm formation in this model system due to high non-specific staining, we used a novel metabolic dye (TTC)-based staining method to measure biofilm formation. In an *in vitro* model system based on media supplementation and replacement with chicken juice, *C. jejuni* biofilm formation was increased significantly in aerobic conditions, when compared to Brucella medium. Using SEM imaging, we show that chicken juice is able to increase biofilm formation in *C. jejuni*, which is linked to the high protein content of the exudate, which may 'condition' the attachment surfaces. As meat juices are found ubiquitously throughout the food chain, they may offer *C. jejuni*, along with other food-chain associated bacteria, a means of increased survival through enhanced biofilm formation. A greater understanding of bacterial survival mechanisms in food matrices will undoubtedly aid in lowering the burden of bacterial contamination of the food chain, and ultimately safer food.

P115. DNA is an essential component of the *Campylobacter jejuni* biofilm extracellular matrix

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The majority of bacteria exist naturally in either single or multi-species biofilms. Biofilm growth leads to increased tolerance to starvation, antimicrobials and survival in food chain relevant conditions. *Campylobacter jejuni* is one of the leading causes of infectious intestinal disease in the developed world. We have previously shown that *C. jejuni* biofilm formation is increased in aerobic conditions, however relatively little is known about *C. jejuni* biofilm formation and its extracellular matrix (ECM) composition. We have investigated the role of extracellular DNA (eDNA) as a component of the *C. jejuni* ECM. Using DAPI staining and GFP expressing *C. jejuni* NCTC 11168, we have shown that eDNA is a substantial component of the ECM. Degradation of eDNA in mature *C. jejuni* biofilms by DNase led to the removal of the biofilm with no loss of *C. jejuni* viability. Strain RM1221, which expresses extracellular DNases, did not form biofilm in static cultures, and co-incubation of RM1221 and pre-formed NCTC 11168 biofilms resulted in the degradation of the mature biofilm. Molecular investigation of this mechanism is currently under investigation. Enzymatic treatment of biofilms in the food chain is becoming increasingly popular and this work highlights the potential effectiveness of these treatments against *C. jejuni* biofilms. Weakening of the ECM leads to dispersal of the biofilm, allowing more efficient sanitisation of food processing equipment and ultimately safer food for the consumer.

P116. Relationship Between Visible Contamination and Campylobacter Contamination on Poultry

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This research tested for a relationship between visible contamination and the counts of campylobacter on carcasses. Reducing the number of visibly contaminated carcases could be a goal if such a relationship existed. Alternatively, intervention methods to reduce Campylobacter contamination might be targeted at these carcasses. Visible contamination consisted of faecal matter or bile and was assessed either post-ev or after inside-outside washing. The contamination was found on the back, neck, and tail. The number of visibly contaminated carcasses was assessed at five plants during eight visits whilst processing 16 batches of birds. At one plant, whilst processing medium sized birds over a 2 hour period, only 1 in 771 birds showed visible contamination. At the other extreme, another plant had 1 in 17 carcasses with visible contamination: the batch lasted

only 2 minutes and consisted of large birds. In one plant, gross faecal contamination was observed around the tail possibly caused by excessive squeezing by the plucker. Considering all plants, the incidence of contamination was least on medium sized birds. At post-ev, four trials showed that carcasses with visible contamination had higher counts than those on "clean" carcasses by 0.5 to 1.2-$\log_{10}$ cfu/g (back/neck skin; $p<0.001$ in all cases). Carcasses with visible contamination post-ev had counts 0.6-log higher than those on "clean" carcasses when tested post-IOW. Correct machinery setting could reduce the number of visibly contaminated carcasses and is good practice that can impact on Campylobacter numbers. Further work will examine whether visibly contaminated birds are a source of cross-contamination.

P117. Differences in Genome Content among two strains of *Helicobacter pylori* isolated from One Patient with Gastritis

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Aims: *Helicobacter pylori* (*H. pylori*) establish a chronic infection in the human stomach. The *H. pylori* population infecting one individual can undergo significant divergence. The aim of this work was to study gene content differences among *H. pylori* strains isolated from one patient with gastritis and investigated the significant of this divergence. Methods: 17 clones from the same patient were analyzed by Multiple Locus Sequence Typing (MLST). 2 clones (wls-5-3 and wls-5-12) were chosen from 2 different clusters and their whole genomes were sequenced. The strains were also tested for their susceptibility to metronidazole. Major findings: 17 clones were divided into 2 clusters in MLST. Few clones were in between, showing partial similarities with both clusters Whole-genome analysis revealed that the two clones showed significant differences. For example, wls-5-12 had excised 27k regions containing energetic components VirD4, VirB11 and VirB4, which mediate early DNA transfer reactions required for bacterial type IV secretion. The two clones are metronidazole- resistance and metronidazole-sensitive respectively. Main Conclusion: These results confirmed that the *H. pylori* population infecting one individual can undergo significant divergence, and more than one strains located in the stomach of same patient might have recombination. Impact of research: Mechanisms and roles in generating strain diversity of one individual can be further investigated.

P118. Prevalence of *Arcobacter* spp. in fresh vegetables from farmers' outdoor markets in Ottawa, Canada.

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The initial aim of this study was to determine the prevalence of *Campylobacter* spp. in raw vegetable produce from local farmers' markets. A total of 406 samples from the farmers' market, and 50 samples from supermarkets were tested using standard methods. Despite the use of microbiological media developed for the recovery of thermotolerant *Campylobacter* spp., the incubation of a portion of the enrichment broths at 37°C rather 42°C, (to favour recovery of stressed cells) resulted in recovery of the related organism, *Arcobacter butzleri*. No *Campylobacter* spp. was identified in any of the 456 samples analysed in this study. *A*rcobacter *butzleri* was isolated from 39 (13%) of the 299 enrichments incubated at 37°C. The majority of vegetables that were positive for *A. butzleri* were leafy green vegetables (14), followed by radishes (6), broccoli (4) and green onion (4). No *Arcobacter* spp. were isolated from the 50 supermarket samples. *Arcobacter* isolates were characterized by MLST, with a subset analyzed by whole genome sequencing. The relatively high prevalence of *A. butzleri* in vegetables sold at outdoor markets may be due to the difference in oxygen requirements and growth temperature of this organism. A more complete understanding of the human health risk posed by *Arcobacter* spp., as well as the epidemiology of this emerging pathogen will be necessary to assess the risk of this organism. More work needs to be carried out to identify the source of vegetable contamination by *Arcobacter butzeri* sold at market.

P119. Dynamics of *Campylobacter* spp. infection in Spanish broiler farms: a longitudinal 18-months study

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A longitudinal study was conducted in five conventional broiler farms during an 18 month period to determine the dynamics of *Campylobacter* infection in Spanish broiler flocks. Weekly sampling was performed in 43 flocks and *Campylobacter* spp. detection was assessed by PCR and culture methods. Samples were obtained from cleaned and disinfected houses prior to chick placement. During rearing, feed and water, chickens (30 cloacal swabs per house and sampling day), and the environments inside and outside the broiler houses were sampled at least once per week. Once all 30 sampled chickens were positive, the whole flock was considered colonized and the sampling finished. Twenty six flocks (60.5%) became colonized during the growing period. No *Campylobacter* was detected prior to chick placement, neither in one day-old chicks nor in the water supply. However, weak *Campylobacter*-positive feed was detected in two flocks by PCR. The earliest a flock became positive was at 14 days of age, while the latest was at 39 days. Once *Campylobacter* was detected in chickens, the whole flock was colonized within 3 to 9 days. *Campylobacter* farm prevalence ranged from 60% to 75% in four out of five farms, while the remaining one showed a lower prevalence (44.5%). In all farms positive and negative flocks were recorded throughout the year, and no clear farm effect or seasonality was observed.

P120. Carry-over of *Campylobacter* spp. during broiler production cycles in Thailand

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Introduction: *Campylobacter* is a major cause of gastroenteritis worldwide. Among warm-blooded animals that can carry *Campylobacter*, poultry are considered as one of the most important reservoirs. The aim of this study was to describe a potential carry-over of *Campylobacter* strains in consecutive broiler flocks in Thailand. Materials and Methods: Two conventional broiler flocks from 2 farms were examined consecutively for 3 production cycles. Cloacal swabs were collected weekly from the first week until slaughter. In addition, caeca were also collected at slaughterhouses. *Campylobacter* was isolated and identified by the direct plating method and multiplex PCR assay, respectively. The confirmed *Campylobacter* colonies were further selected and genotyped by multilocus sequence typing (MLST). Results: Only 4 out of 6 broiler flocks were colonized with *Campylobacter*. Two broiler flocks became colonized at 2 weeks of age with the isolation percentages around 30%. On one farm, broilers were colonized with *Campylobacter* in the first and second cycles. On the other farm, broilers were colonized with *Campylobacter* in the first cycle with the colonization rate ranging from 3% to 46%, but the birds were all negative for *Campylobacter* in the second cycle. In the third cycle, broilers were recolonized with *Campylobacter* at the rate ranging from 73% to 100%. All isolates found in this study were identified as *Campylobacter jejuni*. Discussions: Although the management practices of the two broiler farms are similar, their *Campylobacter* status is different. Genotypes of isolated *Campylobacter* strain are required to prove the carry-over of *Campylobacter* in these broiler flocks.

P121. *Campylobacter* Prevalence during the Broiler Slaughtering Process at the Local Slaughterhouse in Thailand

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Campylobacter is a common bacterial pathogen causing gastroenteritis in humans. The infection in humans is frequently associated with the consumption of *Campylobacter* contaminated poultry products. To better understanding of the distribution of Campylobacter in the local slaughtering plant is need to prevent the contamination. Aim of the study was to estimate the prevalence and risk factors of *Campylobacter* spp. on broiler carcass and contacted surfaces of slaughtering process. The study

was to isolate *Campylobacter* from slaughtering line. There were contacted surfaces (rack, knife, basket, cutting board, evisceration equipment etc.) and contacted carcass samples (water samples, products etc.). 5 samples per each stage were taken. 4 local broiler flocks were sampled. The isolation of *Campylobacter* was done under guideline of modified iso10272 and study on PCR technique. The result has found that mostly contacted carcass and contacted carcass samples were *Campylobacter* positive in different levels of the *Campylobacter* number. However, the carcass package products could not be isolated. The pattern of *Campylobacter* contamination on slaughter line was almost similarity from 4 broiler flocks. PCR results have been under investigation. The results indicated that heavily contamination of *Campylobacter* during the slaughtering was found. The specific intervention method like robus sanitation and hygienic measure of local slaughterhouse on the reduction of the contamination is urgent need.

P122. Design and Evaluation of Vaccines for Control of *Campylobacter* in Poultry

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Campylobacter is the main cause of foodborne diarrhoeal illness in humans in the developed world and it has been estimated that 60–80% of cases are attributed to handling or consumption of chicken. Our previous studies have demonstrated that caecal *Campylobacter* levels in chickens can be significantly reduced by oral vaccination with *Salmonella* Typhimurium *ΔaroA* strains expressing the *C. jejuni* antigen CjaA. We evaluated an attenuated avian pathogenic *E. coli* (APEC) expressing CjaA as a TetC fusion, or TetC, relative to *S.* Typhimurium *ΔaroA* expressing the same antigens. APEC have lower zoonotic potential and a commercially available APEC vaccine (PoulVac® *E. coli*) is used to control avian colibacillosis. In a single trial, neither vaccine decreased caecal *Campylobacter* levels compared to mock-vaccinated controls, though antigen-specific humoral responses were detected. To screen other candidate antigens, the surface-localised and immunogenic *Campylobacter* proteins PorA, FspA1, CjaA, FliD and FlgE2 were cloned, expressed, purified as GST fusions and evaluated as subunit vaccines in chickens. Although decreases in caecal *Campylobacter* counts of up to 1.5 log10 CFU/g were observed in the CjaA and FliD groups at various time points, they were not statistically significant different from controls. Six other candidates have been cloned, expressed and purified and further trials will assess their protective efficacy and seek to reproduce protective effects observed with CjaA and FliD.

P123. 'Within-flock' dynamics of *Campylobacter* colonising a free-range broiler breeder flock; an observational study.

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A broiler breeder flock was studied to determine naturally occurring patterns of colonisation by *Campylobacter* over an extended time period and in a commercial setting where, ultimately, it is hoped that levels of the bacterium may be decreased in order to reduce human infection. A 200 bird cohort was identified by numbered leg rings within a free-range broiler breeder flock of 500 birds. These were sampled individually for *Campylobacter* over 47 weeks. All *Campylobacter* isolates were genotyped by multilocus sequence typing, and statistical analysis was performed using run length encoding and Markov transition matrices in the R software package. The *Campylobacter* shedding rate varied amongst individual chickens from 0–82.1%, with a mean shedding rate of 40.4% over the course of the study. *Campylobacter* sequence types (STs) rapidly and at random within individual birds, with many occasions where *Campylobacter* was undetectable in between times. Rather than STs colonising all individuals within the flock in 'waves' as may happen amongst immature broiler flocks, only a small proportion of the total STs isolated from the flock were detected amongst individual birds. These results indicate there is variation amongst individual birds with respect to *Campylobacter* shedding rate, and suggest that there is natural control of *Campylobacter* dynamics within a flock which could potentially be exploited in designing new intervention strategies.

P124. Phage-*Campylobacter* Interactions on Poultry Carcases.

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The possibility of using bacteriophages to reduce *Campylobacter* numbers by their direct application to foods such as poultry meat or onto environmental surfaces in processing facilities needs further investigation, including the use of kinetic data to design mathematical models. As temperature and atmospheric conditions in these circumstances would prevent growth of *Campylobacter* and replication of phages, the numbers of campylobacters are probably reduced through pre-adsorption of phage which prevents *Campylobacter* growth if conditions should become favourable. The numbers of recoverable *Campylobacter* following application of bacteriophage to contaminated skin was compared with untreated, contaminated controls, at 4°C. Statistically significant reductions in viability of up to 1 $\log_{10}$ between controls and bacteriophage treated samples, were observed when high densities (>7.5 $\log_{10}$ PFU/ cm^2) of bacteriophage were applied. Inactivation occurred within 8 h of application but reductions in cell numbers but did not increase with prolonged incubation (>24 h). The rate of bacteriophage adsorption was calculated for the test phage at 4°C and this data together with the phage treatment on skin will be used to construct a model of phage interaction on chicken skin. By providing a demonstration of the efficacy of phage intervention and providing predictive models for their use in production processes, we will enable regulators to formulate their requirements in light of what is realistically achievable and practicable.

P125. Changes in *Campylobacter* populations in poultry and poultry meat

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Whilst the majority of *Campylobacter* isolates recovered from broiler chicken carcasses post-slaughter are often indistinguishable from those recovered from the live flock, there are significant increases in the diversity of types recovered from carcasses. Strain diversity may have significant implications for the adoption of on-farm interventions or carcass processing strategies. Especially where such interventions achieve only partial control of *Campylobacter* levels and subsequent competition and outgrowth of survivors becomes crucial in mediating the strains that reach the consumer. Farm surveys have demonstrated flocks positive for carriage of *Campylobacter* between 17–21 days post hatching had a single predominant *fla*A-SVR type, however, strain diversity increased within the flock by 31–32 days post hatching. This diversity was detectable using traditional methodology. However, it is highly likely that increased diversity exists prior to this, at levels that are below the level of detection using these methods but can be resolved using higher resolution techniques. Several of the strains thus far isolated from flocks have significant oxygen tolerance when compared even to lab adapted strains such as NCTC11168, surviving at appreciable levels in liquid culture beyond 24 hours. Biofilm formation was also evident and survival of these isolates further along the poultry processing chain may be linked with increased ability to form biofilms, possibly triggered by exposure to oxidative and other stresses. Therefore, identifying strain diversity and population succession is a key requisite to gaining a predictive understanding of the outcomes of current and future interventions throughout the production process.

P126. Role of nutritional status as a determinant in the development of the patients *Campylobacter jejuni* diarrhoea

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Aims: This study was conducted to identify the determinants are infected by *Campylobacter jejuni* in hospitalized under five year's diarrhoeas Children including their nutritional status and severity of campylobacter with diarrhoea. Methods: A case control study was conducted on 206 patients who were admitted in Dhaka Children's Hospital between July 2010 and November 2010. This included 103 children with documented Campylobacter jejuni infection and 103 children as controls who were not infected. Major Findings: A total of 206 samples were analyzed in this study, which showed positive result. Among this patients 92 % are *C.jejuni* and 8% patients are non –campylobacter infected. Socioeconomically condition of the diarrheal patients among **5** *C.jejuni* 0 % patients are poor and 50% patients are semi -medium. Main Conclusion: To address this issue, antibiotic therapy should take into consideration the susceptibility pattern of the pathogen. In addition, the incidence of pathogens in human can be traced primarily to faulty weaning practices and poor personal hygiene. Impact

of the research: It will be possible to identify factors associated with the severity of campylobacteriosis and duration of hospitalization in under-five diarrheal children depending and find the difference in the socio-economic background of their families

P127. Genomics of an unintrogressed *Campylobacter coli* clade 3 strain

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Aims: Genomic characterization of a *Campylobacter coli* isolate with a unique multilocus sequence type and capable of producing gamma-glutamyltranspeptidase (GGT); a characteristic of *C. jejuni* but not described in *C. coli* before. Methods: The whole genome was sequenced using Roche 454 sequencing and was assembled with the Newbler assembler. The scaffold was verified by PCR. Preliminary annotation was performed on the automated RAST annotation server, followed by manual inspection. Results: Phylogenomic analysis showed that *C. coli* 76339 belonged to clade 3 of unintrogressed *C. coli* strains. The *ggt* gene was found among both *C. coli* clades 2 and 3. Bayesian inference of ε-proteobacteria ggt orthologs revealed these potential scenarios a) the *ggt* gene was acquired by an ancestral *Campylobacter* species, initially originating from an ancestral *Helicobacter* species and b) during evolution of both *C. jejuni* and *C. coli* the ggt gene underwent progressive extinction. Also, *C. coli* 76339 carried a lipooligosaccharide (LOS) locus containing a phylogenetically distinct sialyltransferase not present in clade 1. Chromatographic analysis confirmed presence of sialic acid in the LOS of 76339. Moreover, we identified several features which characterized unintrogressed *C. coli* belonging to clades 3 and 2. Conclusion and impact: We propose a novel scenario for the evolution of the accessory *ggt* gene in both *C. coli* and *C. jejuni* species. Also, the presence of sialic acid on *C. coli* LOS may indicate a possible role of this species in post-infectious neuropathies.

P128. Genotyping of *Campylobacter jejuni* by PCR-restriction Fragment Length Polymorphism of the LOS gene.

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We proposed a PCR-RFLP of the LOS gene in order to genotype *Campylobacter jejuni* and compared it with RFLP-PFGE. 23 human strains were tested. RFLP-PFGE was performed using K*pn1* and *Xba*1 enzymes. For PCR-RFLP, PCR was performed in 50 µl, with 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 2.5U of *Pfu Turbo* DNA polymerase, 5 µl of 10X buffer, 0.5 µM of *gal*E1 primer, 0.5 µM of *wla*H3 primer and 5 µl of DNA adjusted to 10 ng/µl. Amplification was done overnight with this program: one cycle of 1 min at 94°C, 35 cycles each consisting of 30 sec at 94°C, 30 sec at 60°C, 15 min at 72°C and a final extension step of 10 min at 72°C. Amplification generated a 9.6 kb amplicon and 10 µl were digested at 37°C with 10 U of *Cf0*1 enzyme, and 10 U of *Rsa*1 enzyme, separately or together. Fragments were separated on a 2% agarose gel. Profiles were analyzed on BioNumerics. PFGE-*Kpn*1 and PFGE-*Sma*1 generated 19 and 18 profiles respectively while RFLP-*Cf0*1 and RFLP-*Rsa*1 generated 7 and 8 profiles respectively. When combining profiles on BioNumerics, 19 PFGE-*Kpn*1-*Sma*1 profiles and 8 RFLP-*Cf0*1-*Rsa*1 profiles were obtained. RLFP with both enzymes in a same reaction generated 12 RFLP-*Cfo*1x*Rsa*1 profiles. Our study showed that PFGE is highly discriminant which is not always suitable for tracking strains; moreover this technique is time-consuming. Our PCR-RFLP of the LOS gene with both enzymes could be a good compromise with an interesting discriminatory power and results obtained in two days.

P129. Determination of Epidemiology of Clinically Isolated Campylobacter strains in Italy by Multilocus Sequence Typing

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Campylobacter jejuni is a leading bacterial pathogen in human gastroenteritis worldwide. Foodborne outbreaks of campylobacteriosis are mainly caused by the ingestion of contaminated raw milk or uncooked chicken meat. We conducted

a molecular characterization of a human epidemic occurred in Piemonte region during the summer in 2012. The aim of this study was to better understand the sources of the epidemic testing potential foci of infection. *Campylobacter jejuni* isolates from 31-gastroenteritis hospitalized patients, 16 raw milk and 9 chicken samples were typed using multilocus sequence typing (MLST) analysis. All samples were taken from the same time point and spatial location. This approach identified 26 different sequence types (STs) and 14 clonal complexes (CCs). According to the pubmlst database, the most prevalent CCs were 21, 206 and 354. At fine scale in 3 instances human cases were linked to chicken source sharing STs 19 and 2863 and in 8 instances human cases were linked to raw milk source sharing STs 122 and 21. MLST revealed the spread of several STs suggesting a multiple source of infections as also demonstrated by the higher polymorphism within *C. jejuni* human isolates. The assessment of the genetic diversity among *Campylobacter* population is critical for our understanding of the epidemiology of this bacterium. Further analyses of isolates from various sources with this approach will permit major advances in understanding their epidemiology and population structure of *Campylobacter jejuni* in Italy. This study confirms the importance of correlating epidemiological investigations with molecular epidemiology to understand the dynamics of infection.

P130. Risk factors for *Campylobacter spp.* contamination in broiler fresh meat production in Italy

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Campylobacteriosis is the most frequently zoonotic diseases in humans worldwide. Incorrect food handling and undercooked poultry meat have been recognised as the main sources of infection. A research project on the identification of risk factor for the presence of *Campylobacter* spp. in broiler meat chain production was carried out in Italy within July 2011-July 2012, divided into eight cycles (two for each season). Two different farms were selected and 220 animals were randomly collected, identified and tested to define the level of contamination of carcasses at different stages of slaughter. Skin samples were collected after each main step of slaughter (bleeding, defeathering, evisceration, washing, chilling). Caeca were also collected. Each sample was tested for detection and enumeration of *Campylobacter spp.* according to ISO methods. *Campylobacter spp.* was isolated in 56.5% of carcasses after bleeding (95%CI 62.9%–50.0%), 30.3% after defeathering (95%CI 36.7%–24.6%), 60.0% after evisceration (95%CI 66.4%–53.6%), 58.4% after washing (95%CI 64.7%–51.8%), 59.3% after chilling (95%CI 65.5%–52.7%). Caeca have showed a prevalence of 79% (95%CI 83.6%–72.9%). Results show the presence of *Campylobacter spp.* in all slaughter phases underlining an increase after the evisceration step caused by intestine ruptures during the production chain. This increase can not be reduced by the following operations of washing and chilling. These data suggest the need of interventions focused on the evisceration step to reduce the risk of *Campylobacter* contamination of poultry meat and the onset of food borne disease for the consumer.

P131. The effect of a synbiotic formula on the gut microbiota of broiler chickens and on Campylobacter population

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With the ban of dietary antimicrobial agents in animal farms, the use of probiotics, prebiotics or synbiotics to reduce pathogen load has attracted a great attention. In particular, Campylobacter jejuni has emerged as a leading bacterial cause of food-borne gastroenteritis in humans, mainly due to poultry products. In this work we evaluated the capability of a synbiotic product to modulate the gut microbiota of broilers in order to obtain a competitive reduction of C. jejuni colonization. The synbiotic treatment (SYN), composed of microencapsulated B. longum subsp. longum PCB133 and a galactooligosaccharide, was administered to broilers for 15 days mixed to normal feed; faecal samples were collected three times during the trial. Considering the ability of PCB133 to reduce C. jejuni in naturally infected broilers, as showed in a previous work, PCR-DGGE analysis was performed to study the microbiota shift during synbiotic administration and after one-week wash-out. PCR-DGGE profiles evidenced that synbiotic consumption induced compositional alterations in the fecal microbiota of treated broilers compared to control animals. Moreover, PCR-DGGE results showed that the band corresponding to PCB133 was detected in all animals and was persistent after the wash-out period. The possible occurrence of different C. jejuni and C. coli genotypes will be also investigated through fla-DGGE analysis. This study allowed to

highlight the positive effect of the synbiotic approach for the modulation of the gut microbiota of broiler chickens, focused on reducing the prevalence of C. jejuni and its presence along the food chain.

P132. Cranberry proanthocyanidin antimicrobial properties: Investigating efficacy against *Campylobacter in vitro* and after colonization in poultry

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Campylobacter is one of the leading causes of foodborne illness and is attributed to consumption of poultry. Currently there are few treatments to reduce *Campylobacter* in poultry. Extracts from the American Cranberry (*Vaccinium macrocarpon*) contain proanthocyanidins which have antimicrobial activity against other foodborne pathogens including *E. coli* and *Salmonella*, but their activity against *Campylobacter* is unknown. The study objective evaluated, *in vitro* and *in vivo*, the efficacy of cranberry proanthocyanidins extract powders containing either low (1%, L-PAC) or high (30%, H-PAC) proathanthocyanidin concentrations. In replicate *in vitro* trials, the lowest concentrations of L-PAC or H-PAC (0.1 or 0.5% wt/vol) didn't reduce *Campylobacter*. At 1% (wt/vol) L-PAC or H-PAC, there was a 1–5 log reduction, and at 2 or 4% (wt/vol) there was at minimum a 5 log reduction in *Campylobacter*. For *in vivo* studies, birds (n=10/treatment group) were given L-PAC or H-PAC at concentrations of 0.5%, 1% or 2% (wt/wt) in the feed starting at day of hatch through day 14. At day 7, all birds were challenged with approximately 2.5×10^5 cfu/mL of *Campylobacter jejuni* by oral gavage and at day 14 birds were euthanized and cecal contents were collected for enumeration of *Campylobacter*. In both trials, cecal *Campylobacter* counts were not reduced by administration of L-PAC or H-PAC in the feed. Although highly effective *in vitro*, further evaluation is needed to determine optimum concentrations of cranberry proanthocyanidins to reduce *Campylobacter* in poultry. Funded in part by the USDA-NIFA-OREI 2011-01955

P133. Heat and Chlorine Resistance of Campylobacter

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Campylobacter is the leading cause of foodborne gastroenteritis in Australia with 30% of cases linked to poultry. The processing sites that have been demonstrated to reduce the carcass level of *Campylobacter* include scalding and immersion chilling. This study aims to assess the effect of commonly utilised heat and chlorine levels on *Campylobacter* survival. A total of 32 *Campylobacter* isolates, from clinical (*C. jejuni*-n=10, *C. coli*-n=3) and poultry (*C. jejuni*-n=10, *C. coli*-n=7) sources plus NCTC11168 and RM2228, were tested for their survival in nutrient broth (NB) at 53.1 and 57.4°C for 5 min. Survival in NB, adjusted to 1.15 ppm chlorine, pH 6.5, was assessed for 20 min. The Weibull model was used to fit all data. Level of decline at 3 min for heating and at 10 min for exposure to chlorine was calculated using the model. After heating at 53°C significant ($P<0.05$) differences were noted between isolates with 10 of the most resistant 11 isolates being *C. coli*. The decline in numbers was greater at 57 than at 53°C and *C. coli* isolates were no longer the dominant heat resistant organisms. Significant ($P<0.05$) differences were noted across isolates after 10 min exposure to chlorine with three from six of the most resistant isolates found to be *C. jejuni*. This work demonstrates that *Campylobacter* have a range of resistance to heat and chlorine parameters commonly seen in Australian scalding and immersion chilling tank fluids. Further work is required to assess the survival of *Campylobacter* on chicken skin under these parameters.

P134. The use of the fluorescent protein, iLOV, as a reporter in *Campylobacter jejuni*

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Green fluorescent proteins (GFP) are widely used to determine patterns of gene expression and track protein distribution within living cells. Recently, a new fluorescent reporter known as LOV has been developed from the *PHOT1*and *PHOT2* genes isolated from *Arabidopsis thaliana*. The LOV protein is a light activated protein kinase that contains two N-terminal Light, Oxygen or Voltage (LOV) domains. The LOV2 domain is derived from the blue-light receptor, phototropin 2

(PHOT2), and was engineered to increase photostability (iLOV). The iLOV reporter shows several advantages over GFP, including a small size of only ~11 kDa, stability over a wide pH range, and efficacy under anaerobic conditions. In this study, we examined whether iLOV could be used as fluorescent reporter in *Campylobacter jejuni* NCTC 11168. The iLOV reporter domain was cloned into the pC46 suicide vector, within the Cj0046 gene coding sequence; Cj0046 is a pseudo gene in NCTC11168. Two plasmids were constructed. In one iLOV expression is controlled by the low level *C. jejuni* promoter, p*metK* (pMetkpC46) and in the other expression is under the control of the *C. jejuni porA* promoter (pPorApC46). Fluorescent level was determined using fluorescence spectrometry and fluorescence microscopy. In addition, the plasmids were transformed into *Campylobacter* and successful recombinants used to investigate the suitability of iLOV. We found that iLOV expression levels matched that predicted from the promoters (Metk, PorA) and fluorescence was constant and quantitative. Also, we show the iLOV reporter in *Campylobacter* is a promising tool for tracking bacterial infection and protein localization.

P135. A molecular study on *cjaC* gene region in *Campylobacter concisus* genome

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Campylobacter concisus is a fastidious hydrogen-requiring, gram-negative bacterium normally found in human oral cavity. It is suggested as an emerging pathogen and has been recently isolated from intestinal biopsies of IBD patients. However, only few of its virulence factors have been thoroughly investigated and its taxonomy was not well established. Thus, this study investigated the molecular characterisation of a genomic DNA region that includes the *cjaC* gene (a putative virulence gene) and its juxtaposed gene no. CCC13826_0962 (that encodes for a hypothetical protein). The *C. concisus* isolates used in our study were 15 strains from faeces of diarrheic children and 16 oral strains form healthy adults. PCR was performed to amplify different parts of the targeted region using several oligonucleotide sets. Our finding indicated that this region can be amplified merely form genomospecies (B) based on oligonucleotides designed according to the *C. concisus* 13826 sequenced genome. Moreover, it was heterogenic across this genomospecies itself as some of the oligonucleotides failed to amplify different parts of the targeted region. Remarkably, all oligonucleotides designed according to *C. concisus* 13826 sequence failed to amplify any part of this region from genomospecies (A) isolates which could be an indication for the unique sequences in both genomospecies. Consequently, our data gave the first molecular insight into the variation of the nominated genomic DNA region in both genomospecies. This PCR amplification method can also be used as a tool for genotyping of *C. concisus*. These findings might be related to the virulence state of this species.

P136. Prevalence of *Campylobacter* and *Arcobacter* species in diarrhoeal faeces from humans in Portugal

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The genus *Arcobacter* and *Campylobacter* belong to the family *Campylobacteraceae*, being *Campylobacter jejuni* and *Campylobacter coli* common causes of bacterial diarrhea in humans worldwide. Currently, other *Campylobacter* species have emerged as potential human enteric pathogens. *Arcobacter butzleri*, *A. cryaerophilus* and *A. skirrowii* have also been associated with human and animal illness. The present study was conducted to investigate the prevalence of *Arcobacter* and *Campylobacter* species in 299 stool samples of patients with diarrhoea, collected from 22 Portuguese hospitals, between September and November 2012. Patients mean age was 27 years old (range 0–99 years); 149 (49.8%) patients were males. Detection of *Campylobacter* spp. in fecal specimens was performed by genus-specific PCR, followed by species-identification by Real-Time PCR with FRET probes, for *C. jejuni*, *C. coli* and *C. fetus*. Detection of *Arcobacter* species was performed in all samples by a Real-Time assay. Overall, *Campylobacter* spp was found in 32.4% of diarrhoeic faeces, with a prevalence of 13% for *C. jejuni*, 1% for *C. coli* and 0.3% for *C. fetus*. Regarding *Arcobacter* spp., four (1.3%) samples were positive for *A. butzleri*, and one (0.3%) positive for *A. cryaerophilus*. The prevalence of *Campylobacter*-like organisms (CLOs) was significantly different between children and adults (40.2% versus 23.5%, $P<0.001$). We underline the high prevalence of CLOs as the aetiological agent of acute gastroenteritis among Portuguese patients, affecting particularly the paediatric age group.

P137. The potential of phage therapy to reduce *Campylobacter* colonization in broilers under consideration of bacterial resistance

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Human campylobacteriosis is the most frequent foodborne zoonosis in many countries. One main cause is handling, preparation and consumption of broiler meat. A reduction of intestinal colonisation of broilers would lead to a considerable decline of human campylobacteriosis. We compared the caecal *Campylobacter* load and the emergence of phage resistant bacteria in a four-phage-cocktail treated broiler group, a single phage treated group and a phage free control eight times in six weeks. Commercial broilers received a *Campylobacter* suspension on day six of live. Three days later, they were treated with the cocktail or the single phage, respectively. The *Campylobacter* load of the individual birds varied considerably. The mean *Campylobacter* load of the treated groups lay in mean 1.3 lg CFU/g below the phage free control. This reduction was statistically significant from day 7 up to day 35 after treatment for both treated groups. The cocktail treatment did not enhance the *Campylobacter* reduction. A phage resistant *Campylobacter* subpopulation emerged in both treated groups. The peak of the resistant subpopulation was achieved three days after application of the single phage and two weeks after application of the phage cocktail. Subsequently, the proportion of the resistant subpopulation decreased continuously. The single phage treatment induced cross resistance against the phages used in the cocktail. Altogether, the cocktail treatment induced slightly more resistance than the single phage treatment. To our knowledge, this is the first log-term investigation of phage therapy comparing phage cocktail and single phage treatment and their impact on phage resistance.

P138. *Campylobacter fetus* subsp. *testudinum* subsp. nov., isolated from humans and reptiles

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The type species of the genus Campylobacter, Campylobacter fetus, has two subspecies: Campylobacter fetus subsp. fetus and Campylobacter fetus subsp. venerealis. Campylobacter fetus strains isolated from reptiles have been previously shown to be genetically divergent from the two known Campylobacter fetus subspecies, with the suggestion that they may represent a distinct taxonomic group. A polyphasic study was undertaken to determine the taxonomic position of 13 Campylobacter fetus-like isolates from humans (n=8) and reptiles (n=5). Isolates were characterized using biochemical tests, growth characteristics, antimicrobial susceptibility testing, 16S rDNA sequencing, genus-, C. fetus-specific-, serotype-specific sap locus-, and sap insertion-PCR, MALDI-TOF MS, pulsed-field gel electrophoresis (PFGE) using SmaI and KpnI, amplified fragment length polymorphism (AFLP) analysis, whole genome sequencing (WGS), optical mapping and DNA-DNA hybridization. Phenotypic characterization, genus-specific- and sap insertion-PCR initially identified all human isolates as type A Campylobacter fetus. Phylogenetic analysis based on 16S rRNA gene sequences revealed that these isolates from humans, along with the isolates from reptiles, formed a robust cluster distinct from the two known subspecies of Campylobacter fetus and other Campylobacter species. Further characterization by MALDI-TOF MS, PFGE, AFLP, WGS, optical mapping and DNA-DNA hybridization confirmed this divergence. This unique cluster of 13 isolates represents a novel subspecies within the species C. fetus, for which the name Campylobacter fetus subsp. testudinum subsp. nov. is proposed.

P139. Molecular epidemiological analysis on *Campylobacter* isolates associated with food poisoning.

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Campylobacter is one of the most common causes of bacterial gastroenteritis in Japan. Nation-wide surveillance of food poisoning cases by the Ministry of Health Labor and Welfare reported 361 cases (2,092 patients) notifications in 2010. However, this represents only a small fraction of the total cases of *Campylobacter*. This report describes the investigation of an outbreak of campylobacteriosis following a meal at a skewered grilled chicken restaurant in Fukuoka city, Japan. The public health center conducted the inspections of the restaurant and identified an association between eating several dishes containing chicken. We conducted a retrospective investigation of the 11 university students who attended dinner at the restaurant with personal interview and microbiological examination. Eight cases had been identified with culture positive for *Campylobacter* but two of them were asymptomatic. Three of the symptomatic persons visited clinics/hospitals but none of them was diagnosed with *Campylobacter* infection. PCR using species-specific primer sets for *Campylobacter* showed that 10 strains isolated from the students were *C. jejuni*. Restriction Fragment Length Polymorphic (RFLP) Analysis using PCR amplified flagellin gene (*fla* A) demonstrated that two distinct patterns were observed among the strains. Random Amplified Polymorphic DNA (RAPD) analysis also showed the same result. Cytolethal distending toxin (CDT) gene sequencing and Multilocus Sequence Typing (MLST) analysis of the strains revealed that the two strains are not related each other. These data suggested that the outbreak might be caused by the food which was contaminated with the two distinct *C. jejuni* strains.

P140. *Helicobacter* DNA in the dorsal patch of the Curaçaoan Long-Nosed Bat *Leptonycteris curasoae* (Glossophaginae)

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Helicobacter spp. colonize the gastrointestinal tract of humans and animals; however, no information exists on the presence of this genus in bats. During the mating season, male individuals of the Curaçaoan Long-nosed Bat deposit body fluids on the interscapular region of the upper back, creating a dorsal odorous patch that is involved in mate choice by females. It has been hypothesized that the odoriferous signal associated with this patch could be the product of microbial activity; however, *Helicobacter* has not been reported as part of the microbiota present in it. The aim of our study was to determine if *Helicobacter* DNA is present in the dorsal patch of the Curaçaoan Long-nosed Bat. The dorsal patches were collected using sterile cotton swabs. Control samples of pelage were taken from the interscapular region of males without dorsal patch and females to determine whether the *Helicobacter* presence was unique to dorsal patches. Fragments of 16S and 23S rRNA genes were amplified by PCR with *Helicobacter* genus-specific primers. *Helicobacter* 16S and 23S rRNA genes were detected in 4 of 4 males with dorsal patch, 3 of 3 males without the patch and 3 of 5 females. Phylogenetic relationship of 16S and 23S rRNA sequences showed that these sequences are closely related to *Helicobacter* species with flexispira morphology. These results demonstrate for the first time the presence of *Helicobacter* genus in Curaçaoan Long-nosed Bats. More studies are necessary to establish the localization of *Helicobacter* genus in the gastrointestinal tract of this natural host.

P141. Whole genome sequencing of *Campylobacter iguaniorum* sp. nov., isolated from reptiles

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Campylobacter species have been isolated from a wide range of hosts, including mammals, birds, and reptiles. Reptiles are not often identified as carriers of *Campylobacter*. Body temperature and metabolism of reptilian hosts is quite different compared to mammals and birds, and *Campylobacter* strains present in reptiles may have specific adaptations. Two strains were obtained from the intestinal contents of reptiles: 1485E, originating from a central bearded dragon (*Pogona vitticeps*), and 2463D, originating from a green iguana (*Iguana iguana*). The whole genomes of strains 1485E and 2463D were sequenced using Roche 454 sequence technology. Scaffold genome sequences were obtained and annotated against the current genome annotation for *C. fetus* subsp. *fetus* strain 82-40. Gap closures were validated with PCR and Sanger sequencing. In addition, the strains were characterized phenotypically with MALDI-TOF. Strain 1485E had a genome size of 1.68 mb, with a 70 kb megaplasmid. For strain 2463D this was 1.81mb and 49 kb, respectively. A difference with the genome of *C. fetus* 82-40 was that both strains were lacking the S-layer region. An average amino acid identity of 78% was observed between the proteomes common to both strains and the closest related known species, suggesting strains 1485E and 2463D represent a novel *Campylobacter* species, which was supported by the MALDI-TOF data. The whole genome sequencing results indicate these strains represent a distinct species within the same taxonomic clade as *C. fetus*, *C. hyointestinalis* and *C. lanienae*. The name *Campylobacter iguaniorum* sp. nov. is proposed for this novel species.

P142. Yerba Mate (*Ilex paraguariensis*): Antimicrobial activity and application as a prebiotic to reduce *Campylobacter jejuni* colonization in broiler chickens

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Dried leaves of yerba mate (*Ilex paraguariensis*) are consumed as a tea beverage in South America. It is know that yerba mate has a beneficial impact on the health of the consumer, hypocholesteroliemic, hepatoprotective, central nervous system stimulant, diuretic. Additionally, yerba mate extracts have been found to have antimicrobial properties against certain foodborne pathogens. The objective of this study was to evaluate the antimicrobial activity of lyophilized yerba mate extracts against *Campylobacter jejuni* and the efficacy of raw yerba mate as a prebiotic feed additive to reduce *Campylobacter jejuni* colonization in a chick model. For in vitro experiments, *Campylobacter jejuni* cultures were suspended in yerba mate extracts and minimum inhibitory concentrations were determined. For in vivo experiments, 60 day-of-hatch chicks were split into 2 groups 1) prebiotic treatment (yerba mate, 0.55% inclusion rate in feed); 2) no treatment (control). At day 3, groups were challenged with *Campylobacter jejuni* (10^7 CFU/ml); at day 10, chicks were euthanized, ceca contents were serial diluted, plated and incubated; after incubation colony forming units were counted and data statistically analyzed. For in vitro, antimicrobial activity was observed at concentrations of 2.49 mg/ml to 19.23 mg/ml depending on strain. For in vivo, the treatment did not reduce significantly the colonization of *Campylobacter jejuni* in the ceca but a numerical decrease was observed compared to the control group. Other possible prebiotic effects on gastrointestinal ecology are currently being investigated, including fatty acid profiles and immune system responses; future studies will evaluate higher inclusion rates.

P143. Population dynamics and genetic profiles of poultry *Campylobacter jejuni* isolates in Slovenia: farm to fork analysis

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Aims: To get an insight into population dynamics and genetic heterogeneity of *Campylobacter jejuni* (CJ) in individual poultry meat production cycles in Slovenia. Methods: Six industrial broiler flocks were examined for the presence of CJ in weekly intervals, to determine an average time of CJ introduction into a flock, speed and extent of colonisation and genetic relatedness of isolates within individual flocks and among different flocks. Same flocks were then CJ-analysed during slaughtering and carcass processing. Neck-skin of individual carcasses was sampled sequentially at predetermined slaughter-line sites, caeca after evisceration step and slaughterhouse environment before, during and after slaughtering. Samples were analysed by isolation method; campylobacters from neck–skin samples were also quantified. Isolates were species-identified and genotyped (PFGE/MLST). Major Findings: On farms, CJ was first detected in birds aged 4 or 5 weeks; one flock remained negative. In positive flocks CJ spread to virtually all animals within a week. PFGE profiles were limited and usually typical for each farm. Carcasses from the CJ-negative flock remained negative also during slaughtering. In other flocks, CJ-contamination was confirmed in all samples. Skin contamination ranged from 1.6×10^3 to $>1.5\times10^4$ CFU/g. Campylobacters were isolated also from aerosol samples. Preliminary PFGE results of slaughterhouse isolates indicate that cross-contamination is possible (multiple pulsotypes detected in i.e. eviscerating machine). Nevertheless, this was not confirmed in carcasses: analyses of neck-skin isolates suggest that carcasses get contaminated by their own caecal/farm pulsotype. Main Conclusion: low CJ genetic heterogeneity on farms; same PFGE-types found in carcasses and respective caeca

P144. Study of the Influence of Agriculture on *Campylobacter* in Recreational Water in Southern Quebec, Canada

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Campylobacter is an important cause of recreational waterborne gastrointestinal illness. The objective of this study was to determine the influence of agricultural activities on the presence, concentration and genetic diversity of *Campylobacter* at beaches used for water recreation. A real time PCR (qPCR) approach was taken to quantify *Campylobacter* in water from recreational beaches in lakes in the Monteregie and Estrie regions of Quebec. Samples were taken from June to August, from 10 beaches in 2011 and 20 beaches in 2012/2013. In parallel, *Campylobacte*r was isolated from the water samples for genetic analysis using Comparative Genomic Fingerprinting (CGF). Site selection of beaches was aimed at maximizing diversity in agricultural land use intensity surrounding the beaches. Farms in the proximal area of beaches were identified using an on-site investigation in order to determine the number and types of farms present. Satellite image classification was used to assess the occurrence and the proportion of agricultural and other environmental determinants of land use. Binomial negative regression showed a significant positive association between agriculture and *C.* spp and *C. jejuni*. Thirteen different CGF types were isolated to date, of which 58% were isolated from humans, animals and water across Canada, whereas 42% were unique water isolates. Quantitative and source data on the presence of *Campylobacter* are paramount in providing a better understanding of potential risks to humans associated with use of recreational water and for better managing of public health to potentially reduce the impact of campylobacteriosis in Canada.

P145. Control of *Campylobacter jejuni* in broilers using a feed additive

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Aims: More than 200,000 human cases of Campylobacteriosis were reported in the EU in 2010 and 50 to 80% of these cases are attributed to the chicken reservoir. According to the EFSA, inclusion of feed additives able to reduce *Campylobacter*

amounts is one of the intervention strategies to consider. This work aimed to determine the effects of a patented ion exchanged clay, used as a feed additive, on *Campylobacte*r contamination in broilers. Methods: Broilers contaminated with *Campylobacter jejuni* isolated from poultry product were fed either with a control diet (additive free) or with supplemented diet using different combinations (durations and doses) of the additive. *Campylobacter* loads were assessed in caecal contents following the decimal dilution method. The comparison between control and treated animals was performed using statistical analysis based on mean and multiple comparison tests. Major Findings: The additive had no effect when used at weak doses (from 5 to 50 ppm) as a preventive action against *Campylobacter*. A curative effect was observed when chickens received higher doses (over 100 ppm) during 3 to 6 days before slaughter. Despite a high variability in *Campylobacter* counts within the treated group, treatments led to a significant ($p<0.05$) mean reduction up to 3 $\log_{10}$/g in caecal contents of treated groups compared to control group. Impact: This investigation provides promising results allowing *Campylobacter* reduction in broilers and consequently the improvement of the food chain safety. Additional work is currently carried out to explain the variability and the mechanism of action of this additive.

P146. Persisting *Campylobacter jejuni* contamination of raw milk on a dairy farm

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Cattle are common carriers of *Campylobacter jejuni* and unpasteurised milk has been recognised as a source of campylobacters in several outbreaks. In November 2012, the origin of a Finnish campylobacteriosis outbreak was traced to a dairy farm. *C. jejuni*, representing an identical PFGE type with the outbreak type, was isolated from cattle and milk. Hygienic measures were implemented including disinfection of the milking machine and milk tank. In order to follow the impact of the measures and improved practices on the farm, sampling of milk and filters from the milking machine was continued. In addition, individual faecal samples from cattle, swab samples from the milking machine and environmental samples from the cattle barn were examined. *C. jejuni* was present in all milk samples at levels from 0.007 to 35 MPN/ml during the four-month sampling period. All isolates from milk and milk filters represented one PFGE type, which was identical with the patient isolate. Apart from the outbreak type, four other PFGE-types were isolated from the faecal samples of cattle. *C. jejuni* was also detected in the samples taken from the feeding table, outlet pipe of washing water and floor drain in the milk room. This study shows that contamination of milk by campylobacters can be persistent and difficult to control, when cattle are carrying the organism in theirintestines and confirms the risk of illness associated with consumption of unpasteurised milk. The investigation continues to find out the cause of persistent campylobacter contamination of milk.

P147. Fly screens in Spanish broiler houses - an initial pilot study

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Fly screens at ventilation inlets, added-on to high level biosecurity, have proven effective to reduce the *Campylobacter* prevalence of broiler flocks to 10.3% during fly seasons over 4 years in Denmark. A replicate of this result in Spain, is the rather ambitious goal of a task of the EU_FP7 financed CamCon project. We will first demonstrate on 2 pilot houses in Catalonia, that fly screens are safe and feasible under the hotter climate in Spain. Second, adequate biosecurity practice will be instituted in 12 study houses around Spain. When effect of biosecurity is confirmed, finally fly screens will be implemented at 6 of the 12 houses. Both pilot houses have transversal ventilation (side wall inlet), in one of the farms combined with tunnel ventilation. This combined ventilation will probably be more common in future newbuilt broiler houses in Spain. For screen material, the lightweight, flexible, UV resistant and durable Phiferglass®Standard-window-and-door screen with 16 x 18 mesh/inch was chosen. At standardized laboratory flowtest conditions this screen material reduced inlet air volume with 22% if mounted directly onto the inlet. Several other (farm related) factors will influence the reduction too, as for example bird stocking density, dust and dirt in the air, individual farm ventilation power, and the local climate. CamCon will monitor the ventilation efficiency at the pilot farms throughout full chicken rearing cycles during spring 2013 to create an algorithm for calculation of the ideal screen surface areas for the 6 Spanish study farms. Major findings and conclusions will be presented.

P148. Fly Screening 101: Technical approaches to ensure ventilation performance without flies

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Studies in Denmark and Iceland since 2004 have reported the effectiveness of fly screens as an add-on to careful broiler house biosecurity to reduce the summer peak of *Campylobacter* in broilers. Screens were custom fit to different ventilation systems (horizontal and vertical). As screens must have fine mesh (<3 mm^2) to prevent entry of *Campylobacter* risk flies (mainly Muscidae), screens can reduce air flow. To compensate for that, the area of screen must be larger than the area of inlets. Required ratios of area of screen material to total area of ventilation system inlets to prevent air flow impedance in the system can be calculated, based on engineering tests to determine air flow impedance curves for each type of screen material to be used. Such tests are based on clean new screen material, to which a margin of safety is added to the ratio to allow for any accumulation of foreign matter on the screen surface. We present specific examples of calculations for safe installations, and on-farm verification tests. Costs of fly screening installations vary with type of ventilation system, broiler house design, cost of local materials, cost of screen material and labour. Examples of cost per broiler house are presented. Some 50 broiler houses have functioned with fly screen over five years, with no noticeable drawbacks for farm management. Currently fly screens are under investigation for feasibility on 2 pilot farms in Spain under the warmer Southern European climate and on 12 farms in the UK.

P149. Control of Campylobacter jejuni in chicken by different phage application strategies

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Campylobacteriosis is the most common form of bacterial food borne enteritis in the world. The disease is predominantly caused by C. jejuni and C. coli, thermophilic species which have a wide range of hosts including chicken, turkey, cattle, other mammals and humans. Commonly recognized risk factors are handling and consumption of contaminated undercooked poultry meat and drinking of unpasteurized milk and surface water. Poultry and poultry meat are regarded as main sources of Campylobacter infection in humans. To reduce the bacteria, several intervention strategies including phage treatment have been investigated. Previously published data demonstrate that phages are generally well suited to reduce the number of pathogens in animals (pre harvest) and on specific products along the food chain (post harvest). Though, the level of attainable reduction largely depends on the target (animal, food), physiochemical conditions and the pathogen. Our work focusses on a pre harvest intervention study that have been undertaken to evaluate the potential of Campylobacter phages for the reduction of C. jejuni in poultry. The study was performed with group II (genome size 185 kb) and group III (genome size 135 kb) phages that were applied individually or in combination (simultaneous and successive application). Here we show that reductions of more than two orders of magnitude were achieved by successive application of group III and group II Campylobacter phages. Thus, a pre harvest application of virulent bacteriophages for the control of C. jejuni in chicken seems to be promising to lower the risk of human infection significantly.

P150. *Do wild brown bears carry Campylobacter species that could cause campylobacteriosis in* humans?

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Campylobacteriosis is a zoonotic disease with a broad range of asymptomatic animal hosts. The role of different wildlife species as reservoirs of *Campylobacter* spp. remains to be determined. The population of brown bears (*Ursus arctos*) in Sweden is approximately 3,500 individuals. During the legal bear hunt up to 300 bears are shot in the fall and the meat is consumed by humans. The purpose of the study was to investigate if *Campylobacter* could be found in feces from wild brown bears. Fecal samples were collected from the rectum of anesthetized brown bears (n=69) as a part of the Scandinavian Brown Bear Research Project and from the colon of bears that had been shot during the legal bear hunt (n=52) in Sweden. The bacteriological analyzes were performed according to the draft protocol ISO 10272 part 1A and 1B (2012). *Campylobacter* spp were found in six of the 121 samples (5%), from two samples collected in May and four in September. All six isolates were identified by phenotypic methods as *Campylobacter jejuni*, which is the species that causes most cases of human campylobacteriosis. PFGE and MLST genotypes of the bear isolates will be compared with isolates from other animals and humans. In conclusion, the prevalence of *Campylobacter* spp. in free-ranging brown bears is low. Our results indicate that the bear population is probably not an important reservoir, but that bear meat or environmental water, contaminated by bear feces are potential sources of infection for humans.

P151. *Campylobacter* in Swedish small scale chicken production. A comparison with findings in conventionally produced Swedish and European broilers.

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Organic and other small-scale produced chicken were sampled and analyzed for detection of *Campylobacter* in ceaca and broiler skin. The project was performed within the Swedish *Campylobacter* program. In Sweden 98% of the chicken are reared conventionally, whereas about 2% of the flocks are reared in organic and other small scale production systems. The aim of the project was to investigate the presence and quantity of *Campylobacter* in small scale produced chickens compared with chickens in the EU baseline study 2008. Swedish flocks in the baseline study were produced conventionally and slaughtered in the major abattoirs. Carcasses and ceacum samples were analyzed from 141 small scale produced flocks, 14 of these came from organic farms. *Campylobacter* spp. were detected in 85 ceacum and 88 carcass skin samples. In the quantitative analysis, *Campylobacter* could be enumerated in 55 of the carcass skin samples and 94% of the samples contained 1–3 log cfu/g. The percentage of positive chicken flocks from Swedish small-scale production 2011 and conventionally chickens from Sweden and EU in the Baseline study.

Pos flocks	**Small scale**	**Sweden Baseline**	**EU baseline**
Ceacum	60.1%	13.2%	71.2%
Carcass skin	62.4%	14.6%	75.8%

A significant difference was found in the *Campylobacter* prevalence between Swedish small-scale and conventionally produced broilers. However, the prevalence was still lower compared with the overall EU prevalence in 2008. Possible reasons for the higher prevalence in small scale production could be outdoor access for the broilers and less strict hygiene barriers in some of the small scale production systems.

P152. Correlation between levels of *Campylobacter* on chicken carcass skin, breast muscle and consumer packed breast fillets

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In the Swedish monitoring program, *Campylobacter* was found in 9.2% of the flocks 2012. The aim of this project was to examine the correlation between *Campylobacter* in chicken skin, breast muscle and breast fillets at retail level. Chicken were selected from producers that had previously delivered *Campylobacter* positive broilers. Five carcasses from each of 25 broiler flocks were sampled at slaughter. Furthermore, 125 consumer packed breast fillets without skin were analyzed from the same 25 flocks. All analyses were performed according to ISO 10272:2006. *Campylobacter* could be quantified in 76 (61%) carcass skins, 3 (3%) underlying breast muscle, and 23 (18%) packed fillets. A significant difference could be seen between chicken skin compared with underlying muscle and consumer packed chicken fillets in number of samples where *Campylobacter* could be enumerated.

Table 1. Results of quantitative analyses of 125 carcass skin, 125 underlying breast muscle and 125 consumer packed chicken fillet samples from 25 flocks.

Campylobacter log cfu/g	Carcass skin samples (%)	Breast muscle samples (%)	Packed fillet samples (%)
<1	49 (39%)	22 (97%)	102 (82%)
1–1,5	10 (8%)	2 (2%)	16 (13%)
1,6–2	25 (20%)	1 (1%)	7 (5%)
2,1–3	37 (30%)	0%	0%
3,1–4	4 (3%)	0%	0%
>4	0%	0%	0%

Conclusions; in most cases where *Campylobacter* was found in carcass skin, *Campylobacter* could not be found in underlying breast muscle. In the cases where *Campylobacter* could be enumerated in breast muscle, the level of *Campylobacter* was about 1 log lower compared with the level in skin samples.

P153. Hygienic processing performance with respect to *Campylobacter* along the processing line within and between broiler processing plants

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The cases of campylobacteriosis in humans attributed to broiler meat consumption are a driving force to establish microbiological criteria for broiler meat. To meet the criteria, measures should be applied along the entire broiler meat supply chain. The application of control measures related to processing hygiene was proposed as the most cost effective strategy in the short term. Although processing steps contributing to an increase or a decrease of *Campylobacter* counts have been determined, it is largely unknown which factors contribute to differences in performance of the operations within a single plant and between plants. The aim of this study is to investigate those factors and their impact on hygienic performance. *Campylobacter* concentration in whole broiler chicken carcass rinse samples was measured in two broiler processing plants in 13 trials. *Campylobacter* status of the flocks was ascertained by farm visits prior to sampling in the plants. The whole carcass rinse method with enumeration on CampyFood Agar (CFA; bioMérieux, France) was used to obtain *Campylobacter* counts on the carcasses. The samples were collected after crucial processing steps such as bleeding, scalding, defeathering, evisceration and chilling. There might be differences in the hygienic performance within a processing plant and between plants. Further data collection and statistical analysis is needed to confirm this statement as well as to find out the impact of factors affecting those differences.

P154. Four years of monitoring of Campylobacter in Dutch broiler meat

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From 2009–2012, the Dutch broiler industry monitored the prevalence and concentration of Campylobacter on breast skin carcasses after cooling. In addition, breast filets were monitored in 2009–10 and caecal samples in 2011–12. Furthermore, all plants performed a risk evaluation as a basis for optimizing their hygienic design and operation. The observed national prevalence of Campylobacter on breast skin samples of carcasses after cooling (i.e. concentration > 10 cfu/g) was 56.1, 54.4, 54.5 and 49.8% in 2009–2012. The proportion of highly contaminated samples (concentration > 10,000 cfu/g) decreased from 1.5% in 2009 to 0.6% in 2012 while the proportion of samples with concentration > 1,000 cfu/g decreased from 9.8% to 8.1%. The concentration on breast skin was correlated with caecal counts, which followed a seasonal pattern. The prevalence and concentration of Campylobacter on breast filets were considerably lower than on carcasses. Plants have made several improvements to the slaughter equipment, e.g. adjustments on the temperature of the water during plucking, intensity of the plucking fingers, frequency of cleaning and disinfection of the evisceration equipment and implementation of extra washing steps. Several plants have invested in new slaughter equipment. A risk assessment model was used to estimate the risks to consumers who prepare broiler meat (cross-contamination in the kitchen). There were considerable differences in Campylobacter occurrence and associated public health risks between individual plants. Partly, this variation is attributable to primary production. Interestingly, the high risk periods in some plants did not always coincide with the summer months.

P155. Inhibition of Campylobacter jejuni by stimulation of Lactobacillus casei growth with peanut fraction

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Aim: As the role of colonic microfloral composition in human health and nutritional value of animal products are getting more attention, the improvement of gut flora with a combination of organic prebiotics and probiotics is a critical area of interest. Modulation of gut flora with synbiotic also improves food safety. This study evaluated the role of peanut fraction in growth of beneficial bacterial such as Lactobacillus casei and their inhibitory role against enteric pathogen, Campylobacter jejuni. In addition with reduction of bacterial pathogen colonization, it may stimulate adaptive immunity by altering cecal microbiome. Methods: Peanut hydrolysate suspension was prepared in water and sterilized by UV irradiation. L. casei and C. jejuni were co-cultured in Bolton broth. Growth patterns for both strains were compared in presence of 0.5% peanut. Findings: We observed that 0.5% peanut suspension had no effect on the growth of C. jejuni but it stimulated growth of L. casei significantly (>1.5 logs) at 24 hr. We also found that in a co-culture of L. casei in presence of 0.5% peanut fraction inhibited the growth of C. jejuni in time dependent manner. The highest inhibition of C. jejuni growth was observed at 72 hr (>2 logs). Conclusion & impact of research: This study has shown the growth stimulation of Lactobacillus with peanut fraction may replace the chemical growth promoters and reduce Campylobacter load in poultry gut. This study is also representing potential value for future in-vivo study which may incorporate probiotic with prebiotic based diet to improve nutritional values.

P156. Media matters: A comparison for the recovery of *Campylobacter* spp. subtypes from integrated poultry production and processing operations

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Aims: Investigations delineating *Campylobacter* epidemiology within broiler flocks to consumers have been conducted; however, an understanding of the fluctuation of *Campylobacter* subtypes through broiler production, processing, and ultimately to the consumer, remains unclear. Furthermore, it is uncertain if the use of varied media and recovery conditions introduces bias on the recovery of *Campylobacter* subtypes. Methods: Three vertically integrated broiler flocks were sampled. For each flock, 10 pooled samples of five fecal droppings were obtained during production, 25 post-chill samples during processing, and 25 exudate samples from processed carcasses stored for two days at 4°C. Sample types were cultured for *Campylobacter* using four different media with four different atmosphere/temperature combinations. Twenty colonies from

each sample type and recovery condition were collected and subtyped using *flaA*-SVR and MLST DNA sequence analyses. Results: Analyses of all trials demonstrated that cultural recovery of *Campylobacter* was similar among the four media, independent of recovery conditions (lowest p-value = 0.267). Subtype analyses revealed the presence of multiple subtypes in all sample types originating from the same flock. Furthermore, distinct subtypes were present in varying ratios within a flock sample type with the dominant subtype fluctuating between sample types. Analysis of variance (ANOVA) was performed to determine if significant bias existed for/against subtypes of *Campylobacter* relative to the media, sample types, and environments tested. Significant biases were observed relative to all three variables. Impact: The method used for *Campylobacter* isolation influences results. Standardization of recovery methods is critical so that bias is eliminated from future epidemiologic investigations.

P157. Cultural and Microbiome Analyses of Commercial Broiler Fertilized Eggs Reveal DNA Signatures for Epsilon Proteobacteria

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Introduction: The role of fertilized broiler eggs in the transmission of food-associated epsilon-proteobacteria to commercial broiler flocks remains unclear. Methods: Fertilized eggs from three commercial broiler breeder flocks at different production ages, young (25 weeks), peak (39 weeks), and old (61 weeks), were simultaneously sampled. Pooled (n=6) gastrointestinal (GI) tracts and yolks were sampled from eggs at 8, 15, and 20 days of age as well as chicks, one-day post hatch. Additionally, egg wash rinsate, obtained prior to dissection, was sampled for the corresponding 20 day-old embryos. Total DNA was extracted, employed for 16S high-throughput amplicon sequencing, and analyzed using QIIME (Quantitative Insights Into Microbial Ecology). Samples were cultured for *Campylobacter* using the Cape Town filtration method and Campy-Cefex Agar. Results: *Campylobacter*-like colonies were culturally recovered and are undergoing analyses. Microbiome analyses revealed *Campylobacter* signatures in all embryonic GI tracts (0.20–1.4% total amplicons) originating from the three breeder flocks. Additionally, 10/12 yolks were positive. DNA signatures for *Arcobacter* were found in embryonic GI tracts (15, 20, and one-day post-hatch) from breeders at peak and old production age. Fertile egg yolks, originating from all breeder flocks, demonstrated signatures for *Arcobacter* at 20 days of age and in post-hatch absorbed yolks. DNA signatures for the family Helicobacteracea were observed in 8 and 20 day-old embryonic GI tracts originating from breeders at peak production. Egg wash rinsate was positive for eggs from both peak and old production breeders. Impact: Microbiome characterization of fertilized eggs will clarify their role in the epidemiology of epsilon-proteobacteria.

P158. Evaluation of a novel in-package ozone-generation treatment system on commercial broiler breast fillets contaminated with *Campylobacter jejuni*

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Introduction: Dielectric discharge barrier-based, in-package ozone-generation is a novel antimicrobial food packaging system that uses atmospheric cold plasma technology to generate ozone inside a sealed package. The efficacy of the ozone generation treatment system was tested against *Campylobacter jejuni* contaminated broiler breast fillets (fillets). Methods: Untreated commercial fillets were initially used to develop and optimize ozone generation. Fillets were placed in Cryovac polyolefin bags and sealed with either ambient atmosphere or a modified atmosphere (65% O_2, 30%CO_2, 5% N_2). The sealed packages were then exposed to several ozonation treatments (variables including voltage applied [75–90 kV] and exposure time [1–5 minutes]). Similarly packaged samples (no ozone treatment) served as non-treated controls. Treated and untreated samples were stored at 4°C for 24 hours. Samples were then rinsed and plated for microbial recovery. Once the system was optimized for reduction of spoilage-associated microorganisms, the efficacy against *C. jejuni* (inoculated at 10^6 cfu/mL) was tested. Results: Treatment conditions determined as most effective for the reduction of spoilage-organisms were 75 kV for 3 minutes under a modified atmosphere. Treated fillets inoculated with *C. jejuni* resulted in a 1.35 cfu/mL log reduction, under ambient atmosphere relative to the control samples, while treatment using the modified atmosphere demonstrated a 2.01 cfu/mL log reduction relative to control samples. Impact: Novel post-packaging intervention systems that effectively reduce food-borne pathogens on fresh raw poultry will provide the consumer with a safer product, thus reducing the risk of food-associated illness.

P159. Monoclonal antibodies against *Helicobacter pylori* γ-glutamyl transpeptidase display neutralizing activity

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Helicobacter pylori produces several virulence factors that contribute to its pathogenesis. One of these is an enzyme γ-glutamyl transpeptidase or GGT (EC 2.3.2.2), a virulence factor of *Helicobacter pylori* found to play a role in colonization, induction of apoptosis, DNA damage and ROS in gastric epithelial cells. However, it is not yet well characterized. In this study, recombinant GGT (rGGT) purified using affinity chromatography through a Nickel chelating column was used to raise monoclonal antibodies (MAbs) in mice. Ten MAbs were produced and classified as two isotypes of the immunoglobulin G (IgG) subclass. Six MAbs were of IgG1 subclass, four were of IgG2 subclass and they recognized the large and small subunit of GGT respectively. MAbs specific towards the small subunit could clearly inhibit GGT activity dose-dependently while those against the large subunit did not show neutralizing activity. A panel of overlapping synthetic peptides was employed to determine the epitope recognized by these MAbs using ELISA. A stretch of 7 amino acid residues was identified as the neutralizing epitope for use to further characterizing the roles of GGT. Furthermore, MAbs against small subunits of GGT were shown to be able to detect low nanogramme level of *H. pylori* GGT by ELISA indicating its potential as a diagnostic agent for *H. pylori.*

P160. Phage F341 requires a motile flagellum for successful infection of *Campylobacter jejuni* NCTC11168

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Phage therapy can reduce the number of *Campylobacter* in chickens, but lowering the number of phage resistant colonies is needed. This may be achieved using a cocktail of phages that utilizes different binding sites for infection. This study therefore focuses on the binding mechanism of phage F341 to *C. jejuni* NCTC11168 to identify the phage receptor. Previous studies have shown that some *Campylobacter* phages require the capsule for infection. Here we found that phage F341 infects a *C. jejuni* NCTC11168 acapsular mutant (Δ*kpsM)* with a 10 fold higher efficiency compared to the wild type, showing that the capsule is not the site of infection. When testing different flagella mutants (Δ*flaAB,* Δ*motA,* Δ*flgP*), F341 no longer adsorbs nor forms plaques. However, F341 adsorbs to flagella and capsular double mutants at wild type levels but with no plaque formation. Thus, phage F341 requires a fully motile flagellum for successful infection. Preliminary results show that F341 adsorb to the *C. jejuni* flagellum and phage-host interaction is currently being investigated using TEM, fluorescence confocal microscopy and by performing flagella binding studies to identify the phage receptor. Moreover, we are genome sequencing phage F341 and aim to identify the receptor binding protein. We speculate that movement of the flagellum is needed for the phage to reach the basal body of the bacterium, and that the initial binding to the flagellum may cause a conformational change of the phage tail that enables DNA injection after binding to a secondary receptor.

P161. Development of spontaneous phage resistance in *Campylobacter jejuni* NCTC12662

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Most bacteriophages infecting *Campylobacter jejuni* have been isolated using NCTC12662 as an indicator strain since it is sensitive to infection by many phages. Here we investigated if *C. jejuni* NCTC12662 develops phage resistance and sought to identify the mechanism of resistance. Interestingly, when *C. jejuni* NCTC12662 was exposed to phage F207 overnight, the culture was dominated by bacterial cells able to grow on a lawn of phage F207, suggesting that resistance occurs at a high frequency. From these plates, a number of colonies were purified, and one (12662R) was further characterized. Surprisingly, 12662R was not completely resistant to phage F207, but showed 100-fold reduced efficiency of plaque formation as well as a reduced plaque size as compared to the wild type. Furthermore, cross-resistance against many phages was also observed. Further analysis showed that *C. jejuni* 12662R is an adsorption mutant. Since we found that phage F207 is dependent on

carbohydrates for adsorption, we compared the ^{1}H NMR and decoupled 1D ^{1}H-^{31}P HSQC spectra of the capsular polysaccharides (CPS) of the wild type and 12662R and observed an attenuated signal for the *O*-methyl phosphoramidate modification, as well as additional unidentified changes in the 12662R CPS. Also, 12662R lost its flagella and became non-motile, suggesting that several changes had occurred in *C. jejuni* NCTC12662 during exposure to phages. Thus, even though *C. jejuni* NCTC12662 is especially phage sensitive, the strain easily becomes phage resistant through general mechanisms of phage resistance in *Campylobacter*, by changes in CPS and loss of flagella and motility.

P162. Inactivation kinetics model for *C. jejuni* on chicken meat under low-temperature storage

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Campylobacter jejuni shows an increased susceptibility to low temperature. Freezing and chilling are effective interventions to reduce the occurrence of *C. jejuni* in poultry meat. The survival rates of three *C. jejuni* strains (ATCC33560, JR0706-2 and ALM-80) inoculated into chicken meat samples were measured at –20℃ and 4℃, respectively. The survival curves of these three strains were determined. The results showed that the survival cells declined by 3.16, 2.87 and 3.14 lgCFU/g, respectively at –20℃ during 55-day storage period. And the survival curves showed that in initial 20 days, the mean inactivation speeds were slow; In 25~45 days, rapid drop occurs, and during the followed period of 10 days, the mean inactivation speeds slowed down. The survived cells were declined by 3.47, 3.35 and 3.51 lgCFU/g, respectively at 4℃ during 10-day storage period. The mean inactivation speeds were 0.347, 0.355 and 0.439 lgCFU/d. There were some differences among inactivation speeds of different strains of *C. jejuni*, but there was no significant difference among the 3 strains ($p>0.05$). Data-fitting MATLAB software was applied to fit the survival rates data. The results showed the established inactivation kinetics function produces good fitness for the 3 different strains at –20℃、4℃ storage. This study revealed the inactivation kinetics characteristics for three *C. jejuni* strains on the chicken meat during the low-temperature storage and provided useful information for *C. jejuni* risk management.

P163. Prevalence, antimicrobial resistance, and genetic characterization of *Campylobacter jejuni* isolated from backyard and commercial poultry in Bangladesh

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Introduction: *Campylobacter jejuni* is one of the most common causes of bacterial gastroenteritis worldwide. We aimed to determine the prevalence, antimicrobial resistance and genetic diversity of *C. jejuni* in backyard and commercial chicken in Bangladesh. Method: A total of 800 rectal swab samples were collected from chicken in backyard and commercial farms and tested for the presence of *C. jejuni*. All *C. jejuni* strains were analyzed by antibiogram, PCR (for 12 pathogenic gens), and pulsed-field gel electrophoresis (PFGE). Result: A total of 333 (42%) *C. jejuni* were identified from 800 rectal swab samples of chicken. Among 333 *C. jejuni*, 204 (51%) and 129 (31%) *C. jejuni* were isolated from backyard and commercial chicken, respectively. The nine genes that encode proteins involved in *C. jejuni* pathogenicity were highly conserved and prevalent (>97%) in both backyard and commercial strains. The isolates from backyard and commercial farms showed highly resistant to ciprofloxacin (78%, 82%) followed by tetracycline (68%, 70%) and ampicillin (42%, 36%). The distribution of multi-drug resistant *C. jejuni* was significantly higher in backyard farms than commercial farm (32% vs 20%; $p< 0.05$). No isolates showed resistance to chloramohenicol, gentamycin and erythromycin. PFGE analysis revealed the heterogeneity of *C. jejuni* isolated from backyard and commercial chicken. Impact of research: The high prevalence of these virulence and toxin genes may contribute to the high prevalence of *C. jejuni* related enteritis in Bangladesh. The scenario of the current antibiotic resistance status of *C. jejuni* may also have implications for the empirical management of campylobacteriosis.

P164. Campylobacter Bacteriophages: Isolation, Characterization and Production

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Campylobacter became an important zoontic agent in recent years and since use of antibiotics as growth promoters was banned in EU, need for novel and efficient intervention strategies has increased. Among these, bacteriophages has been successfully tested and were proven to reduce Campylobacter load in broiler chicken before slaughter in several small scale trials. In present study we isolated and characterized a set of Campylobacter bacteriophages of poultry and pig origin. Further, a scalable production and monitoring for Campylobacter bacteriophage propagation has been developed. Isolated Campylobacter bacteriophages belong to Myoviridae family and can be distinguished by their genome size and DNA restriction profiles. On a molecular level they all require bacterial capsule to initiate infection on their primary C. jejuni host LBA65. Their host range has been investigated using C. jejuni and C. coli human isolates characterized by flaA-RFLP. These bacteriophages tend to lyse C. jejuni more efficiently than C. coli and among former, strains of different geographical origin can be lysed. Propagation of Campylobacter bacteriophages was found to be a bottleneck to efficiently proceed with detailed phage characterization and field tests. Therefore, a production process on a small scale has been developed based on low multiplicity of infection (MOI) infection of bacterial culture. Optical density, pH and dissolved oxygen concentration were monitored to get an insight into the bioprocess dynamics and determine optimal harvest time. This enabled us to compensate for variations in Campylobacter growth and consequently bacteriophage propagation.

P165. Behavior of Campylobacter jejuni LBA65 and Campylobacter Bacteriophage PC5 in Mice model

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Campylobacters as leading cause of foodborne diseases are estimated to cause 9 million cases per year in EU. Because the number of campylobacteriosis cases is increasing since 2005, efficient methods for bio-sanitisation and therapeutic treatments are needed. Among novel antimicrobial agents, bacteriophages have shown to have a therapeutic and diagnostic potential. In the present study we investigated behaviour of C. jejuni and it's bacteriophage in the mice model. Campylobacter jejuni strain LBA65 has been isolated from poultry and it is a motile strain with two polar flagella and capsular polysaccharide. It has been shown that it is sensitive to many bacteriophages and also to flouroquinolone antibiotics, tetracycline and erythromycin. Virulence of C. jejuni LBA65 has been determined in Balb/c mice, infected intraperitoneally with bacterial load ranging from 10^7 to 10^{10} CFU. Infected mice were sacrificed at different time points and the bacterial numbers in the spleen and liver homogenates were determined by plating out and Campylobacter specific quantitative PCR. In addition, local and systemic cytokine response during infection has been analysed. Using the same mice model we examined also stability and distribution of Campylobacter specific bacteriophage PC5. This bacteriophage belongs to Myoviridae family and requires bacterial capsule to initiate infection in LBA65. It's storage stability has been determined over one year long period. In vivo stability has been determined by intravenous infection of mice with 10^8 plaque forming units (PFU) of chromatographically purified bacteriophages. Changes in bacteriophage number has been followed for 12h by infectivity assay and PC5-specific quantitative PCR.

P166. Enhanced Survival Characteristics among *Campylobacter jejuni* Subtypes Obtained from Human Clinical Infections, Livestock Feces, and Surface Water

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Aims: *Campylobacter jejuni* is one of the most important causes of food-borne gastroenteritis. Major sources of infection include undercooked poultry meat, untreated water, and raw milk. Comparative Genomic Fingerprinting (CGF) on 13,500+

Campylobacter jejuni isolates obtained from a variety of sources including human clinical, surface water, and domestic livestock samples suggests that not all genotypes are associated with human illness, which is consistent with a number of previous molecular epidemiological studies. A significant gap in our understanding of campylobacteriosis is whether an association with illness is due to increased pathogenic potential or the enhanced ability to survive the food production environment. Methods: In order to examine the potential association between clinical isolates and enhanced survival characteristics, we chose 36 *C. jejuni* strains from CGF clusters that were predominantly associated with human clinical isolates and from clusters with no clinical association and investigated tolerance to heat, oxygen, and desiccation. Major Findings: Our data indicate differences in survival among isolates, suggesting that tolerance to environmental conditions may play an important role in the epidemiology of campylobacteriosis. Impact: Further research should focus on investigating environmental factors best suited to reducing campylobacter survival in food production.

P167. A Longitudinal Study on *Campylobacter* spp. in Commercial Turkey Flocks in Northwest Ohio: Phenotypic and Genetic Diversity

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Poultry are recognized as a main reservoir of *Campylobacter* spp., but few longitudinal studies have been carried out on commercial meat turkeys. The objectives of this study were to determine the prevalence, antimicrobial susceptibility, and genetic relatedness of *Campylobacter* spp. recovered from three commercial turkey operations in Northwest, Ohio. 810 samples were collected from one-week-old poults to slaughter age, consisting of 750 fecal droppings, and 60 ceca. Multiplex PCR assays confirmed 72% of isolates as *Campylobacter coli*, 5% *Campylobacter jejuni*, 10% *C. coli/C. jejuni* co-existing, and 11% as other *Campylobacter* spp. Restriction fragment length polymorphism of the *flaA* gene (*flaA*-RFLP) subtyping detected 71 types; 62 for *C. coli* and 9 for *C. jejuni* isolates, with most (80%) of *flaA*-types constituting farm homogenous groups. Multilocus sequence typing of 101 selected *Campylobacter* strains resulted in 26 sequence types (STs), consisting of 10 STs for *C. jejuni* strains and 16 STs for *C. coli* isolates. Six new STs; four for *C. jejuni* and two for *C. coli*, were identified. In a subset of isolates tested for antimicrobial resistance, the most common resistance was to tetracycline (96%), followed by azithromycin and ciprofloxacin (44%), clindamycin (41%), erythromycin (20%), telithromycin (11%), nalidixic acid (10%) and gentamicin (9%). All isolates were susceptible to florfenicol. *C. coli* isolates displayed greater resistance than *C. jejuni* to almost all antimicrobials. This study highlights the high prevalence and genotypic diversity of antimicrobial-resistant *Campylobacter* spp recovered from the commercial turkey production systems.

P168. Comparison of genotypes and antibiotic resistance of *Campylobacter jejuni* and *Campylobacter coli* on chicken retail meat and at slaughter

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To determine if certain strains are selected along the production chain we compared the genotypes of isolates from retail meat to isolates from chickens at slaughter and human cases. For this purpose we performed multi locus sequence typing and genetic determination of quinolone and macrolide resistance for 204 *Campylobacter jejuni* and *Campylobacter coli* isolates collected at retail as well as 197 isolates from cecal samples taken at slaughter. Subsequently, we compared both groups to a set of 383 clinical isolates from humans with no history of foreign travel. Species distribution was similar between isolates from retail (69% *C. jejuni*, 31% *C. coli*) and slaughterhouse (74% *C. jejuni*, 26% *C. coli*) but markedly different to clinical isolates (91% *C. jejuni*, 9% *C. coli*). This indicates that the lower proportion of *C. coli* in human patients is likely due to a lower pathogenicity. The genotypes of retail and slaughterhouse isolates were highly similar. Fixation index values (FST), giving an indication of the genetic distance between populations (zero to one), were not significantly different from zero for both species. Both the retail and the slaughterhouse group were also very close to the human isolates with FST values for *C. jejuni* of 0.028 resp. 0.026 and 0.048 resp. 0.044 for *C. coli*. There was no significant difference in antibiotic resistance between isolates from Swiss meat and slaughterhouses, however, isolates from imported meat showed higher resistance. In conclusion, *Campylobacter* genotypes present at slaughter are also those found on retail meat and in many human cases.

P169. Field trials of a bacteriophage-cocktail to reduce *Campylobacter* load in broilers

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Approximately 80% of poultry carcasses are contaminated with *Campylobacter* spp. at slaughterhouse level. *Campylobacter* spp. are the most common pathogens causing foodborne human enteritis. The European Food Safety Authority (EFSA) considers the reduction during primary production as the most effective strategy to reduce the number of cases of human campylobacteriosis. A significant reduction of intestinal colonization of *Campylobacter* in broilers could be achieved by the use of different bacteriophages under experimental conditions. A cocktail containing four *Campylobacter*-specific bacteriophages was tested *in vitro* and *in vivo*. Under experimental *in vivo* conditions a $>\log_{10}$ 2 cfu/g reduction in cecal content was achieved compared to the control and lasted from day 7 until day 21 post application. The cocktail was subsequently tested in three *Campylobacter*-positive broiler farms. On each farm two houses were selected as trial house and control. Fecal samples were analyzed quantitatively before and after phage application for bacteriophage and *Campylobacter*. A dose of $\log_{10}$ 7.5 pfu/animal bacteriophage-cocktail was applied via drinking water. The dose was confirmed in two of the trials via drinking water analysis, while in the third trial a dose of $\log_{10}$ 5.8 pfu/animal was found. The progression of the intestinal *Campylobacter* colonization in the trial groups differed from those of the control groups. In one of the trials no *Campylobacter* could be detected 24h after the phages were applied (detection limit 50KbE/g, P=0.0140). Six days later there was still a significant reduction of $\log_{10}$ 3.2 cfu/g cecal content compared to the control (P=0.0011).

P170. Occurrence of *Campylobacter* spp. in raw cow´s milk in Czech Republic

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A new way of selling chilled unpasteurized milk by vending machines for individual purposes was allowed in the Czech Republic. For monitoring the prevalence of campylobacters, samples collected from 23 vending machines supplied by 14 farms were examined. Samples were taken at least 2 times per year through the whole year period into sterile bottles and transported to the laboratory under at 4 °C within two hours. Samples were examined according the ISO EN 10272 Guideline. Out of 219 samples of raw cow´s milk ten (4.6 %) were positive for the presence of *Campylobacter*. Positive findings came only from three farms and were detected in summer period. For two farms it was only single time detection, in samples from farm C 30 samples were taken and 7 were positive. All isolates were identified by species specific PCR as *C. jejuni*. At the same time, set of *C. jejuni* isolates from patients residing in areas where farm C supplied milk was obtained from clinical laboratories. PFGE analysis of human (35) and milk (9) isolates with *Sma*I restriction enzyme resulted in 24 different profiles (5 in milk and 21 in human isolates). Sixteen (36, 6 %) isolates had a unique PFGE profile. Among 7 isolates from farm C two profiles were distinguished. Both profiles were detected repeatedly in different months and from different sampling sites supplied by farm C. These types were observed also in two patients with campylobacteriosis. Our results demonstrate the link between consumption of unpasterized milk human campylobacteriosis.

P171. Multilocus sequence types and clustered regularly interspaced short palindromic repeats of *Campylobacter jejuni* isolates in organic laying hens in Finland

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Introduction: Production of organic eggs and rearing of organic and free-range poultry is becoming more popular. Our aim was to study the multilocus sequence types (MLST) of *C.jejuni* in organic laying hens in Finland and to associate the MLST types with clustered regularly interspaced palindromic repeat (CRISPR) and pulsed-field gel electrophoresis (PFGE) types. Methods: A total of 146 organic laying hen isolates, collected from 18 different farms in autumn 2003 and spring 2004 and genotyped by PFGE (39 types), were analyzed by MLST and CRISPRs. Results: Fourteen STs were detected among the organic laying hen isolates. Most common were ST-50 (27 %, 7/18 farms), ST-3272 (17 %, 8/18 farms), ST-45 (12 %, 7/18 farms) and ST-356 (12 %, 5/18 farms). ST-45 and ST-50 belong to the clonal complexes ST-45 CC and ST-21 CC that are

frequently isolated from many hosts. ST-3272 was detected often in organic laying hens but has not been common in previous studies proposing ST-3272 might be typical among poultry in Finland. Only one CRISPR type (spacer sequences and number of direct repeats) was identified among isolates of both ST-50 (ST-21 CC) and ST-52 (ST-52 CC). Among isolates of both ST-3272 (UA) and ST-446 (ST-446 CC) two different CRISPR types occurred. ST-45 (ST-45 CC) had the most variable CRISPRs (6 CRISPR types among 7 PFGE types). Impact of the research: Information was obtained about the *C.jejuni* populations in organic laying hens in Finland and the association of CRISPR types with MLST and PFGE types.

P172. Prevalence, genotypes and antibiotic resistance of *Campylobacter jejuni* in Swiss dogs

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To explore the potential zoonotic risk of dogs for human campylobacteriosis prevalence, genotypes and antibiotic resistance of canine *Campylobacter* were investigated. Fecal swabs were collected from 303 healthy dogs and *Campylobacter* isolated by enrichment culture and identified at species level by MALDI-TOF. A prevalence of 6.3 % for *C. jejuni*, 0.7 % for *C. coli* and 5.9 % for *C. upsaliensis* was determined. There was no significant difference in *C. jejuni* prevalence between age groups or between males and females. For genotyping the 20 *C. jejuni* and 2 *C. coli* isolates from healthy dogs were complemented with 114 and 4 isolates, respectively, from routine diagnosis of diseased dogs with gastroenteristis. Multilocus sequence typing (MLST) identified a total of 65 different sequence types (ST) with ST-48 (n=15/11.2%), ST-45 (n=14/10.5%), ST-21 (n=8/6.0%), ST-22 (n=7/5.2%) and ST-441 (n=6/4.5%) being the most frequent. *flaB* typing resulted in 50 different types with the predominant ones being 103 (19.4%), 34 (6.0%), 8 (4.5%), 5 (3.7%) and 36 (3.7%). Genetic determination of quinolone and macrolide resistance based on mutations in the *gyrA* and 23S rRNA genes revealed no macrolide resistant strain, whereas 20.9% of *C. jejuni* and 50.0% of *C. coli* were resistant towards quinolone with no significant difference between healthy and diseased dogs. Comparison of genotypes of canine *C. jejuni* with contemporary human clinical isolates showed a high overlap of both, MLST and *flaB* types, indicating, that despite the low prevalence of *C. jejuni*, dogs should not be ignored as a potential source for human campylobacteriosis.

P173. Investigation of *Campylobacter* outbreaks -the need for close collaboration between authorities, laboratories and other involved parties

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Most cases of human campylobacteriosis are considered to be sporadic, involving 1–2 persons only and the source of infection is seldom found or even looked for. This, together with the lack of a uniform typing system for tracing sources and transmission routes, hamper outbreak investigations where *Campylobacter* is or may be involved. In Sweden, awareness of food and waterborne outbreaks was raised during 2011–2012 when a special Central Outbreak Group was created. The group consists of representatives from the central authorities National Veterinary Institute, National Food Agency, Swedish Board of Agriculture, Swedish Institute for Communicable Disease Control, and National Board of Health and Welfare. The Central Outbreak Group has regular telephone meetings and a standard procedure is followed for outbreak investigations. Much thanks to this initiative, eight outbreaks of campylobacteriosis, caused by *Campylobacter jejuni*, were identified in 2012 with 100–200 exposed cases. Sources for these outbreaks ranged from drinking water and consumption of chicken meat to direct contact with animal sources. Subtyping of isolates from humans and suspected sources by PFGE and MLST has been a useful tool to support the outbreak identification. Also, subtyping has been valuable for understanding the nature of the spread and origin of *C. jejuni* in an outbreak situation.

P174. Correlation of different matrix with *Campylobacter* counts in neck skin of broiler carcasses

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A study was conducted to correlate pre- and post-slaughter *Campylobacter* spp. contamination levels in eight poultry processing plants in Spain (2012). Counts of *Campylobacter* were performed on four pre-slaughter matrices (feathers, crop, ceca, and colon) and a post-slaughter matrix (neck-skin), from 80 broiler flocks (10 flocks/plant). Sampling started once half of the flock was processed, to limit cross-contamination from preceding flocks. For each broiler flock 20 birds were randomly selected pre-slaughter and divided in two groups. One group was used to quantify *Campylobacter* by rinsing method of whole carcass (feathers). The other group was used for ceca, colon, and crop content samples. After slaughter neck skin was sampled from 10 random carcasses by excision method. For each matrix two pools of 5 samples were made, and quantified by conventional culture method (CASA *Campylobacter* plates). Feather count ranged from 1.47 to 6.52 ^{10}Log CFU/bird, cecal counts from 2.43 to 8.40 ^{10}Log CFU/g, colon samples from 1.78 to 8.64 ^{10}Log CFU/g, crop counts from 0.95 to 5.79 ^{10}Log CFU/g, and in neck skin samples from 0.95 to 4.58 ^{10}Log CFU/g. Multivariate regression analysis (SAS 9.3) considering all matrices and the variable ‹slaughterhouse› was performed. Post-slaughter contamination levels were best explained by a final model containing both cecal and feathers counts. However, the model only explained a limited part of the observed variation in neck skin samples (R^2= 0.28, P<.001), indicating that other factors should be considered to allow for proper prediction of final contamination level of broiler carcasses.

P175. *Campylobacter spp.* in poultry environment and its significance for colonization of broilers

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Various animal species, wild birds, rodents and pets are the main reservoir of Campylobacters in broiler farms, however less is known about the ability of this bacterium to survive outside the host, and its role for colonization of broilers. Therefore in this study we aimed to isolate *Campylobacter spp.* from the environment of broiler houses using bootsocks PCR and microbiological methods. In total 323 samples were examined from the three broiler flock rotations in the same broiler house. High *Campylobacter* spp. contamination was found in puddles (50%), and in the farm environment (40%). *C. jejuni* was identified in 25.0% and 31.3% of puddles and environmental samples, respectively. Two out of the three flocks examined were contaminated with *C. jejuni*. The *flaA*-RFLP analysis of 122 *C. jejuni* isolates revealed 12 genotypes in total. Genotype III was dominant in the first flock, and it was also detected in the neighboring flocks, puddles and in the areas around the house from the 9th day of rearing. Similarly, one genotype (IV) was overrepresented in the third flock, which was also detected in the farm environment. In conclusion, we showed that *C. jejuni* remain viable in the broiler farm environment and thus pose a risk to broiler colonization. It can be transferred from the environment into the buildings with shoes, cloths and wildlife, therefore strict bio-security and hygiene rules must be implemented at the farm level. Additionally, hostile environment for pathogenic bacteria must be established in the areas around the house.

P176. Campylobacteriosis in Urban Versus Rural Areas: A Case-Case Study Integrated with Molecular Typing to Validate Risk Factors and to Attribute Sources of Infection

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Campylobacter enteritis is the leading cause of bacterial gastro-enteritis worldwide, and most clinical cases appear as sporadic infections. From July 2005 to December 2007 we conducted a prospective case-case study of sporadic, domestically-acquired *Campylobacter* enteritis and a prevalence study of *Campylobacter* in common reservoirs. The risk of campylobacteriosis was 1.89-fold higher in the rural compared to urban population. Conditional multivariate analysis adjusted for age and sex

identified two independent factors associated with rural area: an occupational exposure to animals (OR=10.6, p=0.032), and water at home coming from a well (OR=8.3, p<0.0001). A total of 851 *C. jejuni* isolates (178 human, 257 chicken, 87 bovine, 266 water, 63 wild bird) were typed by MLST. Among human isolates, the incidence rates of CC ST-21, CC ST-45, and CC ST-61 were higher among the rural than the urban isolates. Based on the MLST results the probability estimates for the source attribution of the human *C. jejuni* isolates were chicken, 64.5%; bovine, 25.8%; water, 7.4%; and wild birds, 2.3%. In our model, chicken was the attributable source for the majority of cases, independent of residential zone, sex and age. The significantly increased incidence in rural compared to urban areas was associated with bovine exposure, particularly among those 15–34 years of age, and likely related to occupation and water consumption from a well. Both bovine and water exposure appeared to contribute to the seasonal variation in campylobacteriosis. These results provide a basis for developing public education and preventive programs targeting the risk factors identified.

P177. Shellfish bear an important number of *Arcobacter* species

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The genus *Arcobacter* includes 17 species, of which some are emerging food and waterborne pathogens. Shellfish have been suggested as a possible reservoir of species. However, only few studies have investigated the presence of *Arcobacter* in this kind of food. The present study aimed to assess the prevalence and diversity of *Arcobacter* spp. in 204 shellfish samples, collected between April 2009 and December 2011, from the Ebro delta (Spain). Detection by m-PCR and by culture was done after enrichment in *Arcobacter*-CAT broth. Cultures on blood agar plates, inoculated by passive filtration, were incubated in parallel under aerobic and microaerobic conditions. Isolates were genotyped (ERIC-PCR) and then identified with m-PCR and 16S rRNA-RFLP methods in parallel. *Arcobacter* was detected by m-PCR and/or culture in 29.9% of samples, obtaining 476 isolates that belonged to 118 different ERIC genotypes (strains). A positive correlation between the positive samples and the increase of the water temperature was found as well as a ca. 10% more positive samples under aerobic than microaerobic culture conditions. The most prevalent species were *A. butzleri* (60.2%) and *A. molluscorum* (21.2%). The latter and *A. ellisii* (2.6%) and *A. bivalviorum* (1.7%) were discovered in this study. Six other species i.e. *A. cryaerophilus* (5.1%), *A. nitrofigilis* (4.3%), *A. skirrowii* (1.7%), *A. thereius* (0.8%), *A. defluvii* (0.8%), *A. mytili* (0.8%) and another potentially new species were also isolated. In conclusion, shellfish are an important source of new species and showed the highest species diversity found up to now from any type of sample.

P178. Prevalence, antimicrobial resistance and multilocus sequence typing (MLST) of *Campylobacter jejuni* and *C. coli* isolated from barnacle geese in Helsinki, Finland

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Introduction: Waterfowl is considered a natural reservoir for zoonotic *Campylobacter* spp. and these birds are potentially a source for human infection either directly or through the environment. Barnacle geese (*Branta leucopsis)* are herbivores and fully migratory waterfowls which reside in urban and suburban areas of Helsinki. Their numbers may reach up to a million birds during migrations. Our objective was to characterize the *C. jejuni* and *C. coli* populations in these birds. Materials and Methods: During 2011 and 2012, 924 fecal samples from barnacle geese were collected from 12 different sites in Helsinki. *Campylobacter* spp. was isolated by cultivation and strains were typed by MLST. A subset of 61 strains was tested for antimicrobial susceptibility against ciprofloxacin, erythromycin and tetracycline. Results: The prevalence of *C. jejuni* in barnacle geese was 23% and 12% in 2012 and 2011, respectively. Two strains of *C. coli* were isolated. Both prevalence and strain diversity was significantly higher ($p < 0.05$) in 2012 compared to 2011. MLST of 152 strains yielded 32 STs of which five were novel. The STs clustered into 7 CCs but 20 STs were unassigned. Tetracycline resistance was found in three strains. No ciprofloxacin or erythromycin resistance was seen. Impact of research: *C. jejuni* STs isolated from barnacle geese were distinct from STs found in *C. jejuni* from human cases in Finland and therefore this reservoir is of minor importance as an infection source for domestically acquired human infections.

P179. A study on prevalence and the diversity of *Campylobacter* spp in chicken farms.

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Aims: To study the prevalence and the diversity of *Campylobacter* spp on farms across Northern Ireland to understand the role of biosecurity. Methods: We sampled 24 farms and collected 171 non-repetitive isolates. Species identification was performed by multiplex PCR. The *porA* gene PCR was performed and the produsts were sequencd. Results: Approximately 60% of flocks tested positive for campylobacter either during the pre or post-thin period. The majority (66%) of isolates were identified as *C. jejuni* and the remainder as *C. coli* (34%). There was a 20% prevalence of *C. coli* in older birds post-thin. Out of the 6 crop cycles, 33 out of 144 flocks showed no changes in the *porA* type when various bio-security measures were implemented . The *porA* gene sequencing showed that the *porA* allele type 932 and 233 were prevalent. A new variant of *porA* was found and is now designated as *porA*- 1691 or MOMP-1547. Conclusion: The identification of campylobacter to species and strain-type level allows comparisons among pre and post- thin time periods. Specific campylobacter strains do not always remain dominant in a reservoir and may be subject to displacement by other clones during the post-thin process. Changes in the population structure of campylobacters will give us a better insight into which are most likely to be present at harvest and hence most likely to challenge humans. Whole genome analysis should help us in deciphering the role of specific lineages by provide insights into inter- and intra- specific spread of campylobacters on farms.

P180. 'Source attribution of *Campylobacter* contamination in the poultry value chains of the UK and Kenya'

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Aims: This project aims at describing the source attribution of campylobacteriosis in poultry value chains in the UK and Kenya in order to provide a basis for comparison in two different institutional settings. Methods: The project will collect data from the feed mill through to the retailer of poultry value chains in Cheshire, UK and Nairobi, Kenya using semi-structured questionnaires. The questions will cover social, economic, behavioural/psychological and environmental aspects to explore disease transmission not explained by poultry meat consumption. An initial risk assessment will identify critical areas of risk for livestock infection and meat contamination. Parallel bacteriological sampling, culture, and genetic typing by Multi-Locus-Sequence-Typing (MLST) techniques will be carried out by a related project. The data collected in the questionnaire and the microbiological work will be the basis for parameters in a fit-for-purpose source attribution model. The model will be verified for its integral logical integrity and validated against the data collected from the two poultry value chains. The model will be used to describe and assess the importance of different points of infection and contamination in the two study sites, comparing the difference between sites. Major Findings and Main Conclusion: (To come at the end of the project) Expected outcomes and impact of the research: The comparative analysis will allow an assessment of the institutional context in determining the transmission of *Campylobacter,* indicating where rules and their enforcement need to be strengthened to achieve cost-effective intervention measures. These results will be of use in both countries of study.

P181. Surveillance of *Campylobacter* spp. in Thai poultry production

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Campylobacter is a leading cause of foodborne diarrheal illness worldwide, with chicken meat as the main source of infection in humans. Although previous studies in temperate countries demonstrated that the prevalence of *Campylobacter* increased during the summer months, the effect of season on *Campylobacter* prevalence in broilers has never been investigated in Thailand. The objectives of this study were to determine the occurrence of *Campylobacter* in Thai poultry production and to investigate the influence of temperature and precipitation on the prevalence of *Campylobacter*. Approximately 250 broiler flocks were examined by collecting cecal samples from slaughterhouses for one year period starting from December 2011 to November 2012. Ten cecal samples per flock were collected and cultured for *Campylobacter*. In addition, the temperature and precipitation were measured during the study period. Around 56% of broiler flocks in Thailand were *Campylobacter* positive. The highest prevalence of *Campylobacter* (82.85%) was observed during the rainy season starting from June to November, while the lowest prevalence of this organism (26.32%) was found during the period of December to February, which is the driest season in Thailand. In tropical countries where the temperature does not drastically alter throughout the year, the occurrence of *Campylobacter* is likely associated with the precipitation rather than temperature. In summary, our study reveals the potential effect of seasonal rainfall on the prevalence of *Campylobacter* in Thai broiler flocks.

P182. Determination of the most appropriate matrix for use in detecting *Campylobacter* in broiler houses.

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Campylobacter spp. are the main cause of foodborne illness in Europe and frequently infect broiler chickens. In order to facilitate a test to detect campylobacters on broiler farms eight sample matrices from broiler houses were compared to determine which would yield the highest number of campylobacters. During on-farm sampling each broiler house was divided into four approximately equal quadrants and one sample was taken in each quadrant. Based on samples from two houses bootswabs, caecal droppings, faeces, and caeca from cull birds were selected for further study. Cloacal swabs, litter samples, ventral swabs and dust sampling was discontinued. Overall twenty five houses were sampled hence 100 of each sample type were analysed. *Campylobacter* spp. were enumerated, and confirmed, based on ISO EN 10272-1:2006. Briefly, samples were plated on modified charcoal cefoperazone deoxycholate agar (mCCDA) incubated at 42°C in a microaerobic atmosphere (85% N2, 10% CO2 and 5% O2, all v/ v) in a MACS workstation (Don Whitley Scientific, Shipley, UK). Caecal droppings and faeces yielded average counts of 6.4 Log_{10} (cfu g^{1}) whilst caeca yielded significantly more ($p<0.05$), i.e. 7.4 Log_{10} (cfu g^{1}). Bootswabs yielded most campylobacters, 7.6 Log_{10} cfu per pair, but these were then suspended in 50 ml of diluent to give 5.9 Log_{10} cfu ml^{1}. Further, 95 bootswab samples were positive but only 89 caeca samples. Based on the relative recoveries of campylobacters, and ease of use, bootswabs were the most appropriate sampling method to detect campylobacters in broiler houses.

P183. *The gyrA* gene: a pertinent target for *Helicobacteraceae* species taxonomy

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Taxonomy of *Epsilonproteobacteria* is based on sequencing of the 16S rRNA gene. However, 16S rRNA gene taxonomy is not sufficiently discriminant in *Helicobacter* species and discordant results were reported for 16S and 23S rRNA gene sequences, suggesting that an alternative scheme to ribosomal genes phylogeny would be useful. The *gyrA* gene encoding the subunit A of DNA gyrase was reported to be an important tool for bacterial phylogeny and was used to identify several organisms including enteric bacteria. In this study, the *gyrA* gene of 55 Helicobacter strains belonging to 25 species was sequenced using chromosome walking and PCR sequenced. Phylogenetic trees were generated by the neighbor-joining method using the entire *gyrA* gene. Preliminary results of the phylogenetic analyses of the *gyrA* gene indicated a good separation of these species, especially between gastric and enterohepatic *Helicobacter* species. Moreover, the phylogenetic analysis of the *gyrA* gene revealed some differences in clustering when compared to the 16S and 23S rRNA gene trees previously reported while a similar clustering to that of the partial *gyrB* gene was observed indicating that the gyrase genes are pertinent target for *Helicobacteraceae* species taxonomy.

P184. Molecular epidemiology of *Campylobacter jejuni* human and chicken isolates from two health units in Ontario

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A population-based study was conducted over two years in the Perth District (PD) and Wellington-Dufferin-Guelph (WDG) health units in Ontario, with an objective of using Comparative Genomic Fingerprinting with a 40 gene assay (CGF40) to investigate the association between human cases of campylobacteriosis and *Campylobacter jejuni* isolates from retail chicken. Laboratory-confirmed human cases of campylobacteriosis were administered a questionnaire to collect risk factor data. Over the same time period and geographical area, retail chicken was sampled according to a representative sampling plan. CGF results were available for isolates from 115 human cases and 718 retail chicken samples. These data were combined with CGF results from a large reference database of *C. jejuni* isolates. Isolates were categorized into types based on > 90% CGF40 fingerprint similarity (CGF-90%). CGF-90% types were categorized as chicken associated (CA90) when the proportion of animal isolates in the given type that originated from chicken in the combined database was at least 80% and was statistically significant ($p<0.05$). Urban cases were significantly more likely than rural cases to be CA90 and there were significantly fewer CA90 cases in the second year of the study. Due to the population distribution in Canada and most industrialized countries, the majority of campylobacteriosis cases are urban dwellers. Therefore, the association between urban cases and chicken associated types of Campylobacter emphasizes the importance of educational and food safety efforts to reduce the impact of Campylobacter from retail chicken on public health. Sources other than chicken may be more important for rural dwellers.

P185. Prevalence of and risk factors for *H. pylori* infection in healthy children and young adults in Belgium.

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Aims: Estimation of prevalence and risk factors for *Helicobacter pylori* (*H. pylori*) infection in children and young adults in Belgium in 2010–2011. Methods: Five hundred and sixteen schoolchildren between 12 and 25 years old were tested for

H. pylori infection using ^{13}C-UBT in different regions in Belgium. A questionnaire was used to evaluate risk factors. Major findings: Eleven % tested positive. In children born in Belgium, with parents from Belgium, 3.2 % tested positive. In children born in a foreign country, 60 % tested positive; if born in Belgium but 1 or 2 parents were from a foreign country, 30 % tested positive. Differences were significant ($p<0.001$). In the multivariate analyses, significant risk factors were staying in a day nursery, a birthplace of child or father outside Belgium, and lower education levels of mother. Main conclusion: In Belgium, the prevalence of *H. pylori* infection in asymptomatic children and young adults is 11.0 %. The most significant risk factor found in this study was origin. Prevalence was 3.2 % in Belgian born children with Belgian parents, 30.0 % if one or two parents where from high prevalence countries and 60.0 % in children born in high prevalence countries. Impact of the research: These results confirm the trend of disappearance of *Helicobacter* in developped countries *and* support the hypothesis of interfamilial spread in Belgium. These findings are important for decisions concerning public health measurements.

P186. Morphological effects of peroxy compounds on *Campylobacter jejuni*

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Gastroenteritis caused by *Campylobacter* infection has been recognized as one of the leading public health problems in most parts of the world particularly in the developed countries. Cell damage and the inactivation of *C. jejuni* from exposure to a number of peroxy compounds were studied by using the transmission electron microscope. Previous data showed that magnesium peroxide and calcium peroxide dramatically inactivated *C. jejuni* in a short period of time (up to 10^6 in 4 hours and 15 minutes respectively) when treated in liquid culture. Given the microaerophilic nature of *C. jejuni,* the reactive oxygen species generated from these two peroxide compounds were thought to be responsible for the inactivation of *C. jejuni*. However, the liquid culture treated with calcium peroxide showed a very alkaline environment, which was at pH 10.0. Therefore, we hypothesized that the inactivation of *C. jejuni* treated with calcium peroxide was predominantly due to the alkaline pH in the liquid culture, and that the inactivation of *C. jejuni* treated with magnesium peroxide was due to the oxidative stress. Transmission electron microscopy confirmed that treatment by these two peroxy compounds harbours different changes in the integrity and cell conformation of *C. jejuni*. Exposure to magnesium peroxide resulted in the irregular conformation and loss of the structural integrity of the cell wall as well as loss of its polar flagella. On the other hand, exposure of *C. jejuni* with calcium peroxide resulted in morphological changes to a round shaped bacterium with intact flagella.

P187. Molecular epidemiology of *Campylobacter* from multiple sources within a Canadian sentinel surveillance site.

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Aims: *Campylobacter* is a significant cause of bacterial enteritis in Canada. Improved surveillance is essential for understanding sources and routes of transmission of this important zoonotic pathogen. C-EnterNet is a Canadian enteric pathogen surveillance system currently operating in two sentinel sites. Methods: *Campylobacter* isolates obtained from enhanced human disease surveillance as well as exposure sources including retail meats, livestock and environmental water from the first sentinel site were examined for predominant subtypes including those implicated in human illness. From 2005–2012, 2327 *Campylobacter* isolates (511 clinical, 1134 farm, 607 retail and 75 water) were obtained from the sentinel site. Isolates were analyzed using Comparative Genomic Fingerprinting (CGF), a high throughput, high resolution subtyping method. Major Findings: CGF types with a low proportion of clinical isolates were often associated with pig manure isolates and were often *Campylobacter coli*. Although many prevalent CGF subtypes with a high proportion of clinical isolates were associated with retail chicken isolates, we also found significant evidence for an association with cattle isolates or a combination of both sources. These data are consistent with previous studies that have found a correlation between high incidence of campylobacteriosis and cattle density. Main conclusion: We have exploited the high epidemiological specificity of CGF to

explore possible linkage between cattle and poultry among clinically relevant *Campylobacter* subtypes. Impact: Cattle are known to be significant reservoirs of *Campylobacter* and elucidating their role in the epidemiology of campylobacteriosis will be essential to mitigation strategies aimed at reducing the burden of illness.

P188. Prevalence of *H. pylori* infection among Japanese junior high school students

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Background and study aims: To clarify the prevalence of *H. pylori* infection among Japanese junior high school students, serum *H. pylori* antibody and pepsinogen were measured. Methods: As a part of an annual school health examination to develop the educational system for preventing of lifestyle-related diseases during adolescents, serum *H. pylori* antibody and pepsinogen were measured at two junior high schools in Nagano prefecture locating at the central part of Japan in 2012. After written informed consent had been obtained from both students and their parents, students underwent a blood test and completed a self-administered questionnaire including information on the presence of abdominal pain, allergic diseases and family history of *H. pylroi* related diseases. Results: Of the 95 students included (51 girls, aged 11–15 years), 3 (3.2%, three girls) were seropositive. PG1 level of them was 65.7, 80.9, 21.1ng/ml and PG1/2 ratio was 1.9, 2.2, 3.1, respectively. The degree of obesity was —19.8 ~ —9.2**%**. One of them was complicating with iron deficiency anemia. One of them had symptoms of recurrent abdominal pain. All of them had any allergic disease. None of them had obvious family history of *H. pylori* related diseases. Conclusion: The prevalence of *H. pylori* infection is dramatically decreasing among Japanese junior high school students.

P189. Recent acquisition of *Helicobacter pylori* by Baka Pygmies

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Both humans and the gastric pathogen *Helicobacter pylori* originated in Africa, and have been intimately associated for at least 100,000 years. Three of the seven geographically distinct *H. pylori* populations are indigenous to Africa: hpAfrica1, hpAfrica2, and hpNEAfrica. The oldest and most divergent population, hpAfrica2, evolved within San hunter-gatherers of southern Africa. Anticipating the presence of ancient *H. pylori* lineages within all hunter-gatherer populations, we investigated the prevalence and population structure of *H. pylori* within Baka Pygmies in Cameroon. We tested *H. pylori* from 77 Baka, using 101 non-Baka individuals from neighboring agriculturalist populations as controls. Unexpectedly, Baka Pygmies were much less commonly infected (20.8%) than the non-Baka (80.2%). Multilocus sequence analysis did not identify Baka-specific lineages, and most isolates were assigned to hpNEAfrica or hpAfrica1. The population hpNEAfrica, a marker for the expansion of the Nilo-Saharan language family, was divided into East African and Central West African subpopulations. Similarly a new hpAfrica1 subpopulation, identified mainly among Cameroonians, supports the idea of eastern and western expansions of the Bantu language. An age-structured transmission model shows that the low *H. pylori* prevalence among Baka Pygmies is achievable within the timeframe of a few hundred years and suggests that demographic factors such as small population size and unusually low life expectancy make the maintenance of population-level *H. pylori* infection precarious. The Baka were thus either *H. pylori* free or lost their ancient lineages during past demographic fluctuations. They almost certainly acquired their extant *H. pylori* through secondary contact with their non-Baka neighbors.

P190. Getting *Campylobacter* out of the poultry food chain by enhancing the knowledge base of the poultry industry.

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As early as 1981 poultry was identified as a reservoir and source of human campylobacteriosis. The importance of this source has now been confirmed and the scientific understanding of the transmission and infection process has increased substantially. Despite this knowledge the reduction of human campylobacteriosis remains an ongoing ambition for public health authorities. Recent analyses, undertaken by an EFSA Working Group, have focussed attention throughout the poultry farm-to-fork chain but emphasise that the production of *Campylobacter*-negative flocks at the farm level would have the added benefit of addressing transmission routes via environmental contamination as well as through the handling and consumption of poultry meat. On-farm control has proved extremely challenging for the industry. In the current absence of effective specific interventions, such as vaccination, biosecurity is the only on-farm approach available. The provision of relevant information to the poultry industry has long been considered key to the successful exclusion of *Campylobacter* from conventionally-reared broiler flocks. Nevertheless, this strategy has had limited success and it has been suggested that we, the "scientific experts", have largely failed the industry by giving mixed messages, false hopes and irrelevant or jargon-laden information. This presentation contends that many of these communication problems arise from differences in the motivation and technical awareness at various points throughout the information chain. Considering these differences, and calling on many years of experience supporting all stakeholders, we will recommend strategies for the delivery of evidence-based information to the poultry industry aimed at increasing the production of *Campylobacter*-negative flocks.

P191. Prevalence and antimicrobial resistance of Campylobacter isolates from poultry in Plateau state, Nigeria.

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A study was designed to determine the prevalence and antimicrobial resistance of *Campylobacter* species isolated from poultry in Plateau state, Nigeria. Three hundred and sixty (360) cloacal swab samples were randomly taken from 18 poultry flocks in Plateau State, Nigeria and analyzed for the presence of *Campylobacter* species. Out of the 18 flocks tested, 13 (72.2%) were positive. Out of the 360 samples tested, 129 (35.8%) were identified as *Campylobacter* species using biochemical tests; with 105 (81.4%) as *C. jejuni* and 24 (18.6%) as *C. coli*. The isolates were further confirmed using polymerase chain reaction (PCR). Sixty five (65) of the *Campylobacter* strains (53 *C. jejuni*, 12 *C. coli*) were randomly selected for antimicrobial resistance test using the agar disc diffusion technique. Ten antimicrobial agents were tested. Of the 65 *Campylobacter* isolates tested, 51 (78.5%) were resistant to one or more of the ten antimicrobial agents while 14 (21.5%) were susceptible to all the antimicrobial agents tested. Resistance to ciprofloxacin (55.4%) and nalidixic acid (55.4%) were the most common, followed by sulphamethoxazole/trimethoprim (47.7%) and erythromycin (47.7%), tetracycline (43.1%), and azithromycin (29.9%). All the isolates were susceptible to clindamycin. *C. coli* isolates displayed significantly higher rates of resistance ($P < 0.05$) to tetracycline, erythromycin, azithromycin and chloramphenicol than did *C. jejuni*. Thirty four (52.3%) of the isolates were multiresistant, being resistant to 3 or more antimicrobial agents. The results of this study highlight the need for antimicrobial resistance surveillance and rational use of antimicrobial agents in food animals in developing countries, especially in Nigeria.

P192. Composting poultry manure by fly larvae (*Musca domestica*) eliminates *Campylobacter jejuni* from the manure

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Aims: The house fly, *Musca domestica* (Md) is an important carrier of zoonotic agents. *Campylobacter jejuni* is one that may be transmitted to broilers by *Md*. In the lifecycle, adult flies deposit eggs in manure where the fly larva develops. We followed the lifecycle from egg to adult in manure containing *C.jejuni*. Methods: Md larvae were transferred to fresh poultry manure containing 10^{+8}/g *C.jejuni*. The number of *C.jejuni* in the manure, inside larvae, in pupae and in the adult fly, was followed

by direct CFU counts and preenrichement culture. Manure samples without larvae served as controls. Major Findings: The composting action of larvae actively reduced the number of *C. jejuni* in manure. In the manure with larvae, the number of *C.jejuni* was reduced to less than 100 CFU/g within 3 days, and could not be detected by enrichment culture after 4 days. In control samples without larvae the number CFU was only reduced to 10^{+6}/g *C.jejuni* on day 3, and could still be detected by enrichment on day 7. The number of *C.jejuni* inside the larvae resembled the number in manure, thus all were negative on day 4. Pupated larvae and adult flies were tested and they were all negative using enrichment culture (detection limit 10 CFU/g). Impact of the research: Fly larvae may accelerate the degradation of *C.jejuni* in poultry manure without being carrier itself. By composting using fly larvae the manure may both be reduced in weight and sanitized from *C.jejuni*, thus reducing risk of contaminating the environment.

P193. Survival of *Campylobacter Jejuni* and *Campylobacter Coli* on Retail Broiler Meat Stored at -20, 4 and 12°c, and Development of Weibull Models for Survival.

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The survival of *Campylobacter jejuni* and *Campylobacter coli* strains isolated from broiler meat was investigated and modeled on retail breast meat at -20°C, 4°C and 12°C. Boneless, skinless broiler breast meat was evenly inoculated on all sides with a phosphate buffered saline suspension of colonies from modified Campy-Cefex agar supplemented with 5% lysed horse blood. Storage at -20°C for 84 days resulted in reductions of 2.88 ± 0.59 and 2.75 ± 0.51 log CFU/g for *C. jejuni* and *C. coli*, respectively, with the most significant reduction appearing in the first 48 hours, while storage at 4°C for 14 days resulted in reductions of 0.83 ± 0.41 for *C. coli* and 2.01 ± 0.48 log CFU/g for *C. jejuni*. Although survival of *C. jejuni* and *C. coli* was similar at -20°C, survival rates were significantly lower ($P < 0.05$) for *C. jejuni* than for *C. coli* at 4 and 12°C. The trend at 4°C where *C. coli* survival was greater than that for *C. jejuni* was highly amplified at 12°C, with the time for a 1 log reduction at 12°C ranging from 4.75 days in *C. jejuni* species to 7.65 days in *C. coli* species. Kinetic data were fitted to the Weibull model, and when ≥70% of the residuals were in an acceptable prediction zone from −1 (fail-safe) to 0.5 (fail-dangerous) log units, the model was considered to have acceptable performance. A difference in survival was observed between the two strains of *C. jejuni* (Cj 1065 and Cj 971) tested.

P194. A real time model of *in-vivo Campylobacter* populations within chicken caeca: applications for bacteriophage host dynamics

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Campylobacteriosis continues to be a leading cause of bacterial enteritis. Interventions to reduce *Campylobacter* contamination of poultry at farm and abattoir currently available in the EU are ineffective. *Campylobacter* specific bacteriophages can reduce colonization levels in broilers within 24 hours, therefore phage therapy offers a potential form of biocontrol. Precise timing of treatment may be important to achieve optimal *Campylobacter* reduction pre-slaughter. Studies suggest spatial considerations need to be given to Campylobacter colonization and in particular phage replication in the chicken gut. To examine these possibilities in situ we have developed procedure to deliver phage and monitor Campylobacter and phage replication in intestinal sections of anaesthetized chickens. This procedure has enabled us to establish that: 1. caecal compartments behave as independent sample sites with 1 $\log_{10}$ differences evident in pre-colonized birds; 2. Campylobacters colonizing the caeca show marked growth post sampling of ligated sections (presumptively the organisms are under oxygen limitation but are certainly not limited by nutrient availability); 3. The time frame for phage replication is 6–8h, which longer than that observed for similar densities of bacteria in culture. This is to our knowledge the first reported viable model of real time *in-vivo Campylobacter*-bacteriophage dynamics. We also note that the ability to maintain stable anesthesia in chickens for these time periods offers the prospect of examining competitive *Campylobacter* colonization in specific intestinal sections that have not been subject to prior stochastic population changes.

P195. Characterization of *Campylobacter* spp isolated from birds in Antarctica and sub-Antarctica

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Campylobacter spp. isolated from birds in Antarctica and South Georgia in 2012 were analyzed regarding species, genotypes, and antimicrobial resistance patterns. The aim was to gain knowledge about *Campylobacter* characteristics of strains among wild birds in the Antarctic and sub-Antarctic region, and to investigate relationships with *Campylobacter* in humans and domestic animals. 41 strains of *Campylobacter* spp isolated from 15 gull (*Laridae*), 7 giant storm petrel (*Procellariidae*), 7 skua (*Stercoraiidae*), and 12 sheathbill (*Chionididae*) samples, were included in the study. Species identification was done by biochemistry, mass spectrometry, and PCR techniques. The identified *Campylobacter* species were: *C. jejuni* (18), *C. lari*, (12), *C. peloridis* (4) and 7 hippurate-negative isolates provisionally termed as *C. lari* - like. All *C. jejuni* isolates were susceptible to erythromycin, tetracycline, streptomycin, gentamicin, and fluoroquinolones, whereas 7 *C. lari* and 5 *C. lari* - like strains were resistant to fluoroquinolones. Eight strains, 3 *C. lari*, 1 *C. lari* - like and the four *C. peloridis* strains, did not grow in the panels for antimicrobial resistance testing. Whole genome sequencing will determine species of the *C. lari*-like strains and give detailed genetic characterization of the *C. jejuni* isolates. In a previous study of macaroni penguins, more or less resident in sub-Antarctica, *C. jejuni* of MLST type ST-45 was found. This MLST type is also common in humans with campylobacteriosis and in food producing animals. The present study will add to our understanding of what types of *Campylobacter* are carried and possibly spread by the birds studied herein.

P196. Distribution of *C. concisus* genomospecies 1 and 2 in faecal samples from healthy volunteers and diarrhoeal patients

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Where appropriate isolation or detection methods are used, *Campylobacter concisus* is often found to be as common as the well-established pathogen *C. jejuni* in cases of human diarrhea. The role of *C. concisus* in disease is however controversial, since it may also be found in healthy patients. We have previously determined the presence of *C. concisus* in faecal samples from healthy volunteers (53%) and diarrhoeal patients (47%) using a pan-Epsilonproteobacterial PCR-DGGE approach. Taxonomically, *C. concisus* is best described as a species-complex containing several phenotypically indistinguishable, but genetically distinct taxa referred to as "Genomospecies", of which there appear to be two dominant types. It has been suggested that these types differ in their pathogenic potential. We validated the accuracy of a nested PCR assay to differentiate the two major genomospecies; and applied this assay to samples in which *C. concisus* had been detected, to assess their distribution among healthy (n=10) and diarrhoeal (n=22) faecal samples. GS1 was detected in 5 diarrhoeal samples; GS2 detected in 6 of each sample type; and both Genomospecies detected in 4 healthy, and 11 diarrhoeal samples. Our results do not suggest a predominance of either Genomospecies in either type of faecal material, pointing to a more complex relationship between *C. concisus* and human disease.

P197. The analysis of intra-familial transmission using multilocus sequence typing of Helicobacter pylori

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H. pylori infection mainly occurs in childhood. The exact transmission route is not clear but intra-familial transmission is considered to be most common in Japan. We have analyzed intra-familial transmission of *H. pylori* using MLST. *H. pylori* antigen positive feces from three children and their family members were selected from Sasayama study. In addition, *H. pylori* strains isolated from the patients with gastroduodenal disease and their family members who live in Sapporo or Asahikawa were analyzed. Total DNAs were extracted from fecal specimens or *H. pylori* strains isolated from gastric biopsy specimen or juice. The DNAs of samples were amplified by PCR using *atpA, efp, mutY, ppa, trpC, ureI* and *yphC* genes. The products of PCR were analyzed by direct sequencing. The obtained direct sequencing result was submitted to the MLST web-site, and the closest allele typing and the candidate(s) of the sequence type (ST) were determined in each sample. In the analysis of three families using faecal specimens, mother-to-child transmission was suspected in at least 2 of 3 families, and father-to-child transmission was suspected in one family. In the analysis of other five families using *H. pylori* strains, mother-to-child transmission, child-to-child transmission and husband-to-wife transmission were observed. All index children had the family member(s) identified as a source of *H. pylori* infection. These results demonstrate that MLST of *H. pylori* DNA is a useful tool for detection of intra-familial transmission. The infection within family members seems to be a risk factor for transmission of *H. pylori*.

P198. Characteristics of patients from whom different *Campylobacter* species are isolated in the United States, Foodborne Diseases Active Surveillance Network (FoodNet), United States, 2010–2012

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Campylobacter causes approximately 1.3 million illnesses in the United States each year; little is known about demographic and risk factor differences for infection with different *Campylobacter* species. FoodNet conducts population-based surveillance for *Campylobacter* infection in 10 sites; isolates are speciated at state health departments and the Centers for Disease Control and Prevention. We examined patient demographic, clinical, and travel information by *Campylobacter* species. A total of 19,950 *Campylobacter* infections were reported to FoodNet in 2010–2012. Among 7,483 (38%) speciated isolates, 90% were *C. jejuni*, 7% were *C. coli,* and 2% were *C. upsaliensis*. Compared with *C. jejuni* patients, *C. coli* patients were more likely to be Asian (7% vs. 3%; $p<0.0001$), Black (7% vs. 4%; $p=0.0154$) and to have traveled internationally in the 7 days before illness (22% vs. 16%; $p=0.0002$); *C. upsaliensis* patients were more likely to be female (58% vs. 43%; $p=0.0023$) and to have a blood isolate (4% vs. 1%; $p<0.0001$) and were less likely to have traveled internationally in the 7 days before illness (2% vs. 16%; $p<0.0001$). *C.jejuni* patients were more likely than *C. coli* and *C. upsaliensis* patients to report fever and bloody diarrhea. The differences in patient characteristics we report are similar to those described in other countries. Because only a small percentage of isolates are speciated, and they may not be representative of all cases, it is difficult to evaluate species-specific trends, risk factors, and prevention strategies. Improvements in laboratory capacity for *Campylobacter* speciation are essential for better understanding *Campylobacter* infections.

P199. Human *Campylobacter fetus* subsp. *testudinum* infections

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Campylobacter fetus is an uncommonly reported species that typically affects immunocompromised, pregnant, or elderly persons and can cause severe infections. Two subspecies, *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*, associated with sheep and cattle, have previously been described. The first isolation from a human of *C. fetus* with markers of reptile origin was reported in 2004. Subsequent characterizations of this case, additional human cases, and reptile isolates identified this as a newly proposed subspecies named *C. fetus* subsp. *testudinum* sp. nov. (Cft). We summarize epidemiologic information from nine known human cases of Cft. Illness onsets ranged from 1991 to 2010 and patients resided in four US states. All patients are male and their median age is 73 years. Five of six patients with known race are Asian. Cft was isolated from blood (5 cases), hematoma, pleural fluid, bile, and stool. Five of 5 patients with information provided had underlying chronic illness, 6/6 were hospitalized, and one died. Three Asian patients and the non-Asian patient reported eating traditional Chinese dishes. Some of these patients also ate eel, frog, and turtle soup. One patient reported contact with a turtle that had diarrhea. In summary, Cft causes an invasive opportunistic infection in adults, particularly of Asian origin. Infection may be related to exposure to traditional Asian foods as well as reptiles. Enhanced public health surveillance and laboratory testing, and surveys of the prevalence of Cft in reptiles are needed to better understand the epidemiology and burden of this newly-proposed subspecies.

P200. Prevalence of *Helicobacter pylori* in a Malaysian population.

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The human populations in the Southeast Asian region have been shaped by numerous migrations. The prevalence of *H. pylori* in Malaysia is reflected by those ancient human migrations from India, China, and the ancestors of the Thai people. We studied *H. pylori* prevalence in 254 serum samples from adults Malaysian individuals. *H. pylori* and CagA serology were determined by ELISA, as previously described. We found a low prevalence of *H. pylori* (18.5%) and even a lower CagA prevalence (8.4%). Of the 254 serum samples, 64 were obtained from an underserved native population from Malaysia. *H. pylori* and CagA prevalence was nearly identical in this group when compared with an affluent group of Malaysians of different ethnic origins. We did not observe any differences related with age, and the only major difference was a higher prevalence in *H. pylori* and CagA that we observed in individuals with Indian ancestry, compared to those of Chinese or Malaysian origin. This study confirmed previous reports of low prevalence of *H. pylori* in Malaysia using conventional detection methods, and also demonstrated the influence of ethnic origin on *H. pylori* prevalence.

P201. Production and Characterization of Hen Egg Yolk Immunoglobulins Against *Campylobacter jejuni*

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Poultry meat products are sources of *Campylobacter jejuni* for humans. Chickens usually become colonized after the 2nd week of age, suggesting the presence of natural barriers against *C. jejuni* such as the transfer of maternal immunity (IgY). The objectives of this study were to characterize the presence and specificity of antibodies against *C. jejuni* in egg yolks and to evaluate different protocols to maximize IgY production and efficiency. To do so, 40 SPF laying hens were separated in

4 groups: control, orally infected by *C. jejuni*, subcutaneously injected with bacterines or with *C. jejuni* outer membrane proteins (OMP). The immunizations and inoculations were based on the same mix of four well characterized strains and were performed at 16 weeks of age. Immunization boosters were given at 21 and 29 weeks of age. Eggs were collected and the IgYs were extracted from the egg yolks using a chloroform-based protocol. Total IgY and anti-*C. jejuni* antibody levels were determined by ELISA. Immunoblots against OMPs and total proteins of *C. jejuni* strains were performed to compare the specificity of antibodies obtained from each production methods. Inhibition of *C. jejuni* motility and the bactericidal effect of the immunoglobulins were performed to evaluate the neutralizing capacities of the IgYs produced. Those upcoming results will allow to select the best method for antibody production to test an egg powder enriched with immunoglobulins against *C. jejuni* as a feed additive at the farm level to control *C. jejuni* intestinal chicken colonization.

P202. Automated *De Novo* Genome Assemblies and Epigenomes of Bacterial Pathogens

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Understanding the genetic basis of infectious diseases is critical to enacting effective treatment. Bacterial sequencing typically maps sequence reads against known reference strains. Such resequencing informs on the spectrum of single-nucleotide differences relative to the reference, but can miss numerous forms of variation known to influence pathogenicity: structural variation (duplications, inversions), acquisition of mobile elements (phages, plasmids), homonucleotide length variation causing phase variation, and epigenetic marks (methylation) that influence gene expression to switch bacteria from non-pathogenic to pathogenic states[1]. Sequencing methods which provide complete, de novo genome assemblies and epigenomes are necessary to fully characterize infectious disease agents in an unbiased, hypothesis-free manner. We have developed a new paradigm for microbial de novo assemblies in which SMRT sequencing reads from a single long insert library are used exclusively to close the genome through a hierarchical genome assembly process, eliminating the need for a second sample preparation, sequencing run, and data set. We have applied this method to achieve closed de novo genomes with accuracies exceeding QV50 (>99.999%) for samples including Campylobacter, H. pylori, E. coli, Salmonella, Listeria and Neisseria. The kinetic information from the same SMRT Sequencing reads is utilized to determine epigenomes. Approximately 70% of all methyltransferase specificities we have determined to date represent previously unknown bacterial epigenetic signatures. With relatively short sequencing run times and automated analysis pipelines, it is possible to go from an unknown DNA sample to complete de novo genome and epigenome in about a day.

[1]Srikhanta et al. (2010) *Nat Rev Microbiol* 8: 196–206.

P203. Molecular characterization of *Campylobacter* isolated from Thai broiler production chain

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Campylobacter is an important foodborne pathogen that commonly found in broilers. In Thailand, information of *Campylobacter* in chicken meat production cycle is still limited. To fulfill this knowledge, we aimed to describe genetic relatedness of *Campylobacter* in Thai broiler production by conducting longitudinal studies on five broiler production chains. Sample collection was performed chronologically at breeder flocks, hatcheries, broiler farms and slaughterhouses. To detect *Campylobacter* along the poultry production line, cloacal swab samples from breeders, environmental samples from hatcheries as well as chicken related samples and environmental samples from broiler farms and slaughterhouses were collected and cultured for *Campylobacter*. Multilocus sequence typing (MLST) was performed to describe genetic relatedness of *Campylobacter jejuni* isolated from each stage of broiler production chain. All breeder and broiler flocks were tested positive for *Campylobacter,* while all samples collected from hatcheries were *Campylobacter* negative. Several types of environmental samples (e.g., transport crates, eviscerating equipments and chilling water) and meat products collected from slaughterhouses were also contaminated with *Campylobacter*. At least four sequence types including ST-45, ST-354, ST-574 and ST-2409 were identified. The ST-574 was the predominant sequence type in broiler flocks, while ST-45 and ST-354 were more common among the isolates from slaughterhouses. In summary, the genetic diversity of *Campylobacter jejuni* in Thai broiler production

chain was revealed. Additionally, the similarity between sequence types found in this study and those previously reported among human isolates in Thailand was observed.

P204. Quantification of Campylobacter in broilers at different stages of slaughter in a commercial processing plant

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Campylobacter has been implicated as a major cause of food-borne illness worldwide. Fecal contamination of carcasses during slaughtering process represent the main source of Campylobacter for fresh poultry meat. The objective of this study was to determine the association between the concentration of Campylobacter in chicken feces at pre slaughter stage with post slaughter contamination of poultry carcasses. From 220 individually identified chicken, representing 8 different batches, skin samples were collected at each of the main slaughtering stages (bleeding, defeathering, evisceration, washing, chilling). From each animal, caeca were also collected. Each sample was tested for detection and enumeration of Campylobacter spp. according to ISO methods. A Spearman's correlation coefficient was calculated to ascertain correlation between Campylobacter concentrations in caeca content and on relative carcasses after evisceration stage. No correlation was observed (R=0,002, p-value=0,518) between level of Campylobacter concentration in caeca content and on carcasses. This result suggests that other factors as cross contamination due to slaughter practices and processing techniques play a more important role in contributing to the risk of poultry carcasses contamination.

P205. Characterization of Presumptive *Campylobacter* Strains Submitted to the CDC Reveals a Diverse Array of Species Associated with Human Disease in the United States

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While *Campylobacter jejuni* is the primary cause of human campylobacteriosis, the public health significance of the other species remains to be determined. Presumptive *Campylobacter* strains (n=864), primarily hippurate negative strains, were submitted voluntarily by state health departments to the National Enteric Reference Laboratory, CDC specifically for species identification between 2000 and 2012. The species identified, the associated source, and the demographic data was reviewed for 762 isolates submitted. The isolates were identified by phenotypic and molecular methods. Of the 864 isolates submitted, 762 (88.2%) were identified as belonging to the Genus *Campylobacter*, *Helicobacter*, or *Arcobacter*. The remaining 102 isolates (11.8%) were either nonviable or were other bacterial genera. The species distribution was *C. jejuni* (23.1%), *C. coli* (22.7%), *C. upsaliensis* (13.1%), *C. fetus* (8.9%), *C. lari* (9.1%), *Helicobacter species* (5.8%), *Arcobacter butzleri* (2.5%), and other *Campylobacter* species (2.6%). *C. fetus* (81.2%) and *C. lari* (72.6%) were primarily isolated from males. *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* were primarily isolated from stool. *C. fetus* was isolated primarily from blood (76.6%), followed by *C. upsaliensis* (13.6%). The diverse array of species identified among strains submitted to the reference laboratory demonstrates that other *Campylobacter* species and related organisms also cause human disease. Since the sampling of isolates was not random, the species distribution found in this study does not reflect the true prevalence of the different species identified in the general American population. The public health significance and epidemiology of the non-*C. jejuni* species needs to be elucidated.

P206. Dominance of two clonal complexes ST-353 and ST-21 among *C. jejuni* isolates from children campylobacteriosis cases in Lithuania over one year period

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Introduction: Campylobacteriosis is the most common reported gastrointestinal infection in many countries. The Centre for Communicable Diseases and AIDS has reported the increase in number of *Campylobacter* infection cases from 18.3 to 25.8 for 100000 inhabitants per year in Lithuania during the period 2006–2011. Methods: In total 126 campylobacteriosis cases of children from 2 months to 18 year old were confirmed at the Microbiological laboratory of Kaunas Clinical Hospital in Lithuania within one year period starting from September of 2011. *Campylobacter jejuni* isolates were genotyped with Multilocus Sequence Typing (MLST) to investigate genetic diversity of *C. jejuni* isolated from children clinical cases in Lithuania. Results: *C. jejuni* was identified in 105 children isolates and *C. coli* in 21, respectively. In overall, MLST genotyping of *C. jejuni* isolates revealed 42 different sequence types (STs). Thirty nine STs, representing 102 (97%) isolates, were assigned to 19 previously described clonal complexes (CC). Three STs were unassigned to a previously identified CC. Eight isolates were identified as a new STs previously unreported in the PubMLST. Dominant clonal complexes ST-353 (27 isolates) and ST-21 (23 isolates) were dominant among *C. jejuni* isolates found in children and accounted for 47.6% of all isolates. Impact of research: Although *C. jejuni* clonal complex ST-353 is rare cause of infection in human in Europe, we found that this CC is even more often found in infected children then ST-21 in Lithuania. Acknowledgements: This research was funded by a grant (No.SVE05/2011) from the Research Council of Lithuania.

P207. The role of bacterial taxis in biofilm formation of *Campylobacter jejuni*

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Campylobacter jejuni is the primary cause of food-borne illness in the developed world. In the environment, a biofilm mode of growth is thought to aid survival in otherwise stressful conditions such as atmospheric oxygen, which is harmful to this microaerobic organism. We have previously shown that biofilm formation is stimulated by aerobic conditions, but the mechanisms governing this response remain unknown. Here we have investigated the influence of chemotaxis and energy taxis, the coupling of motility to environmental sensing, on biofilm formation in *C. jejuni*. Using a static growth glass slide assay, wild-type *C. jejuni* was shown to form a thick biofilm at the air-surface interface, which could be visualised by crystal violet staining and composite image analysis. Towards the base of the slide, discrete clusters of adhered cells were observed. A software tool was developed to quantify crystal violet-stained cells to measure biofilm porosity. Inactivation of the CetAB energy taxis system resulted in a much reduced biofilm at the air-surface interface, and abolished formation of discrete adhered cell clusters. Absence of the chemotaxis protein CheA also impaired the formation of air-surface interface biofilm. Finally, biofilm formation was affected by the energy taxis protein CetZ (Tlp8): a *cetZ* mutant showed reduced biofilm porosity, while a CetZ-complemented strain formed a thick structured biofilm. Motility in known to be important for biofilm formation, and these results further suggest that environmental sensing and taxis are important factors for robust biofilm formation in *C. jejuni*.

P208. Campylobacter Infection in the Channel Islands

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Background: *Guernsey and Alderney* Between 2000 and 2011 rates of campylobacter infection were more than double those in England and Wales. Previous unpublished investigation of this phenomenon was confined to Jersey so during 2012 a questionnaire was administered to all notified cases on Guernsey and Alderney. Isolates from 28 indigenous infections were speciated and typed. Comparisons were made with data and isolates from indigenous infections in Jersey. Methodology: *Guernsey and Alderney* The telephone questionnaire covered occupational details; travel history; contact with animals; food,

water and milk consumption and sources; recreational activities, hobbies and environmental exposures. Results and Discussion: *Epidemiology Guernsey and Alderney* Eighty four (62%) of the cases were interviewed. There was no evidence of outbreaks or common source infections. The aetiology of campylobacter infection in Guernsey and Alderney appears to be multi-factorial. A culture of outdoor activity may contribute to high infection rates but relevant factors may be under-reported. This was also a risk factor in the Jersey study. Microbiology: The majority of isolates was *C. jejuni*. Isolates from Guernsey exhibited a lower frequency of ciprofloxacin resistance than those from Jersey otherwise phenotyping was unremarkable. Multilocus sequence typing showed isolates of 11 ST-complexes amongst the Guernsey isolates and 14 amongst the Jersey isolates. When individual sequence types were considered a similar diversity was found among isolates from both islands. Nothing immediately outstanding was identified, though the difference in the frequency of ciprofloxacin resistance between the islands was interesting.

P209. A proteomic study of the host specificity and stress responses of Campylobacter

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The determination of how the processing of food affects contaminating *Campylobacter* phenotypically and the extent to which source food species corresponds to genotypic and phenotypic traits will contribute to an understanding of the extent to which different strains adapt and survive through the food chain and to cause disease in humans while others pose no threat to public health. The two stranded proteomic approach is examining the epidemiological aspects of host specificity and the effects of environmental conditions in the food chain. In order to survive in the environment and then through the food chain, *Campylobacter* needs to endure a large variety of stresses such as heat, cold, oxidative, osmotic, bile salt, UV and acidic stresses. Specific genes have been identified in the literature which are involved in these stresses, many of which are global response mechanisms regulated in response to a variety of different stresses, not simply single pathways for single stresses. By generating and utilising our Representative Strain Collection of *Campylobacter* isolates, from clinical and known host species sources, the effect of relevant stresses (heat stress (scalding/de-feathering); cold stress (chilling/freezing); lactic acid wash (cleaning carcass)) can be applied to elucidate relative fitness across a broad spectrum of *Campylobacter* strain types. This was determined using survival and protein expression measurements. A low-resolution proteomic approach has allowed the rapid identification of *Campylobacter* to a species level (*C. jejuni* & *C. coli*) and specific protein biomarkers enabling this are being identified.

P210. Source attribution of human campylobacteriosis cases in Sweden during 2012

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Introduction: Source attribution analysis (SA) has primarily been used to investigate the origin of human salmonellosis and campylobacteriosis. The method uses strain classifications to assign human cases to a source that contains the matching strain. A study is ongoing, with the objective to perform SA on Swedish cases of campylobacteriosis during 2011-11-01 to 2012-10-31. This paper presents the project, data collection and preliminary results. Methods: A total of 979 human isolates of Campylobacter were randomly selected for the study from 3412 domestic cases during the study period. Multilocus sequence typing (MLST) was used to classify the isolates. Samples from Swedish poultry (n=257), cattle (n=189), pigs (n=190), sheep (n=417), surface water (n=510), wild birds (n=500), companion dogs (n=184), imported food at retail (n=77) and from the origin (n=100) were collected, cultured for Campylobacter and will be classified by MLST. This data will be used to estimate the primary sources of Swedish human campylobacteriosis. Results: The MLST typing will be completed in July 2013, at which point we can run SA. Presently, the human isolates typed (n=786) fall into ST-21 complex (42%), ST-45 (15%), ST-48 (12%), ST-677 (6%), ST-257 (4%) and 21% are other complexes. Poultry, cattle and sheep follow a similar distribution but the water and wild bird isolates preliminarily include few isolates from the above ST complexes. Impact: The study will contribute to describing the epidemiology of Campylobacter in Sweden and to compare the reservoirs of Campylobacter in Sweden to those found in previous investigations in other countries.

P211. The prevalence and antibiotic resistance of *Campylobacter coli/jejuni* strains isolated from patients with diarrhoea and from chicken carcasses

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Intro: Contrary to many other EU countries, in Poland, *Campylobacter* is rarely isolated from humans with diarrhoea because the diagnostic test is not routinely performed. *The aim of the study was to determine* the prevalence and antibiotic resistance of *Campylobacter coli/jejuni* strains isolated from patients with diarrhoea and from chicken carcasses. Methods: The studies were performed between July 2009 and June 2013 in West Pomeranian Region of Poland. 1218 stool samples were taken from hospitalised patients and outpatients, 77 samples from chicken carcasses. For isolation of *Campylobacter* selective media CCDA, was used with identification performed using Multiplex-PCR method. Susceptibility to erythromycin and ciprofloxacin was determined with E-test method. Results: *Campylobacter* strains were detected in 6.6% of diarrhoeal stool samples, most often in children below 3 year (9.8%). The positive samples were found most often in June and September. Chicken carcasses were contaminated with *Campylobacter* in 58.4% of cases. *Campylobacter jejuni* was the dominant species in patients (91.3%) and in poultry (88.9%) samples. 98.8% human strains and all chicken strains were susceptible to erythromycin. Resistance to ciprofloxacin was detected in 82.4% of human and 57.8% of chicken strains. Impact of research: *Campylobacter jejuni* is an important human pathogen responsible for gastrointestinal disorders in each age groups especially in children below 3 years and mainly in summer months. The high percentage of resistance to ciprofloxacin among clinical *Campylobacter* strains is probably associated with the use of fluoroquinolones in veterinary medicine. Erythromycin can be still used in the treatment of more severe cases of campylobacteriosis.

P212. The effect of DNA extraction technique and physiochemical parameters on the presence of epsilon-proteobacterial genetic signatures within a commercial broiler house: A microbiomic- and qPCR-based investigation

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Introduction: Commercial broiler houses are typically considered a single environment along the poultry production chain, but diverse physiochemical parameters persist throughout the house. Due to the different matrices and environmental parameters that exist within a house, optimization of molecular techniques to determine the microbial component of these samples, including the presence of epsilon-proteobacteria, is essential. Methods: A commercial house containing ~20,000 59-day old broilers was divided into 4 distinct areas, and litter and fresh fecal/cecal droppings were sampled from each area. The DNA was extracted from all samples using three different methods (physical-based, chemical-based, hybrid) and deep sequenced using the Illumina MiSeq platform. Additionally, all samples were subjected to physiochemical and nutrient analyses. Results: The prevalence of genetic signatures for *Helicobacter* were ~10X higher than for *Campylobacter* throughout the different areas of the poultry house, with the lowest prevalence for each being found in the highest moisture areas (near the waterer/feeder lines). The chemical-based DNA extraction method yielded the highest prevalence of *Campylobacter* and *Helicobacter* genetic signatures for both the litter and fresh fecal/cecal samples, with the physical-based method yielding the lowest prevalence values. The accuracy of the prevalence estimates for *Campylobacter* were validated by comparing those values to qPCR data targeting total bacteria (16S) and *C. jejuni* (*hipO*). Impact of Research: Understanding the effect of the matrices (including physiochemical parameters) and analytical techniques is paramount to studying the dynamics of epsilon-proteobacteria in broiler house studies, and this serves as baseline broiler house microbiomic data for future live production studies.

P213. *Campylobacter* positive samples and hygienic scoring on slaughterhouse equipment and catching crews

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Slaughterhouse equipment frequently arrives visibly contaminated on farm. Catching crews often are not employed by the integration itself but by independent sub-contractors and they abide by markedly different quality standards. Does hygienic scoring of slaughterhouse equipment on farm and hygienic scoring of different catching crews lead to different levels in the results? In total 18 depletion processes, carried out by 15 different catching crews were monitored for presence of *Campylobacter* and for defined hygienic criteria. A total of 214 swabs was taken and evaluated according to the method described in ISO 10272-1:2006 by two different laboratories. Hygienic scoring was carried out in parallel to bacteriological examination. Eighty % of all positive samples were found in slaughterhouse equipment on farm. It was demonstrated that catching crews were associated with *Campylobacter* contamination. Furthermore a large gap between different hygienic statuses of single catching crews was evident. Another weak point was the hygienic status of farm vehicles, especially in cleaning. From these results the following recommendations concerning slaughterhouse equipment were given: cleaning process of slaughterhouse equipment needs to be examined much more closely and markedly improved. It would be prudent to include the epidemiologic context into these examinations. As the observed standards differed considerably between catching crews it was advised to design guidelines to address demonstrated weak points and to grant a reliable standard. These standards are briefly presented. Ideally integrations could recommend farmers to employ catching crews only from an accredited pool of crews.

P214. *Campylobacter* prevalence and concentration in self-contained poultry supply chains

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Our target was to represent *Campylobacter* epidemiology in poultry not on a nationwide basis but in a self-contained supply chain. We looked at *Campylobacter* being carried through from environment, to primary production until slaughter. This was combined with a detailed analysis of processes influencing prevalence at each single step. Results were used to tailor prevalence reduction concepts for self-contained integrations. Environmental sock samples were taken on farm to assess prevalence. Crates and catch team boots were swapped, both at thinning and at final depletion to identify involvement of slaughterhouse equipment arriving cleaned on farm and catching crews before coming in contact with chicken on farm. Caecum samples were taken in the slaughterhouse to reflect on farm and transport influence on prevalence as well as concentration. Finally, neck skin samples acquired before chilling were intended to reflect the slaughter process up to that point in the manner of a black box. Caeca and neck skin results were semi-quantified as well. Furthermore using *fla*A typing, strains were followed through the above described sampling steps. Problematic steps included contamination of crates on farm and the contamination of the immediate vicinity of the broiler house prior to thinning and final depletion procedures. A detailed analysis of current practices in an integration as opposed to desired procedures revealed considerable room for improvement. It furthermore highlighted the importance of regular training and auditing to achieve the desired compliance. This is a fundamental step in assuring success of target orientated measures in a self-contained supply chain.

P215. Impact of Transport and Holding Time on *Campylobacter* External Contamination on Broilers

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Broilers originating from *Campylobacter* positive batches can carry high numbers of *Campylobacter* on their feathers and breast skin at the beginning of the slaughter line. In order to define the source of this external contamination, we aimed to determine the effect of transport and holding procedures on the external *Campylobacter* contamination of broilers, colonized with *Campylobacter*. From nine *Campylobacter* positive flocks, feathers samples (n=6) and underlying breast skin samples (n=6) were collected from broilers before transport to the slaughterhouse and at the beginning of the slaughter line (after bleeding). *Campylobacter* was quantified by direct plating on CampyFood Agar® (bioMérieux SA, France) plates. Results

revealed a high variability in external *Campylobacter* contamination of broilers before and after transport. In most cases, feathers were higher contaminated than breast skin samples, both at farm and slaughterhouse level. A maximum increase in contamination with 2.6 and 3.6 log cfu/g was observed during transport and holding time for breast skin and feathers, respectively. *Campylobacter* was even recovered at the slaughter line on breast skin and feathers from a flock from which the birds were not externally contaminated (LOD=10 cfu/g) at farm level. High, externally contaminated birds at the start of the slaughter process may influence the *Campylobacter* load on carcasses after slaughter. Reduction of external *Campylobacter* contamination of broilers entering the slaughterhouse by controlling the external contamination at the farm level and by optimizing the transport/holding conditions might be essential for decreasing the *Campylobacter* load on broiler carcasses after cooling.

P216. Quantification of the *Campylobacter* Carcass Contamination during the Slaughter of *Campylobacter* Positive Batches.

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Controlling of the *Campylobacter* contamination during slaughter is crucial for the reduction of public health risk. In order to evaluate the effect of the slaughter on carcass contamination the study aimed to quantify the *Campylobacter* contamination on broiler carcasses originating from *Campylobacter* positive batches throughout the slaughter process. Four Belgian broiler slaughterhouses were visited at least four times, following the slaughter of a *Campylobacter* positive batch. At each visit, 6 carcasses at 7 locations at the slaughter line and 6 intestinal packages were collected. *Campylobacter* was quantified by direct plating on CampyFood Agar® (bioMérieux SA, France) plates. Results showed that broilers sampled during all visits (n=18) carried high numbers of campylobacters in their caeca ($\geq$ 7.6 log cfu/g). Additionally, a high variability in *Campylobacter* carcass contamination within batches, between batches in the same slaughterhouse and between slaughterhouses was observed. The slaughter of batches with low external contamination at the beginning (< 3 log cfu/g breast skin) led to an increase of contamination after plucking and evisceration. However, this effect was not visible when initial contamination was high. The impact of a combined washing and cooling step varied between batches and slaughterhouses, with the highest decrease of 1.2 log cfu/g in one slaughterhouse. The capability of slaughterhouses to control the initial external contamination level, the cross contamination during plucking and evisceration and the effectiveness of washing and cooling steps what has an important impact on the *Campylobacter* carcasses contamination after cooling.

P217. CamCon - Multi National Study of *Campylobacter* risk factors on broiler farms, Part one - Denmark and Norway

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Many Campylobacter risk factor studies have been carried out at the national level. This study, which is part of the EU financed CamCon project, provides the first set of results from a multinational risk factor study carried out in six EU countries. As the first step, data from Norway and Denmark were analyzed. Farm data were collected through a standardized questionnaire survey, while Campylobacter data were obtained through existing surveillance programmes for Campylobacter in broilers. Farm data from 107 Danish and 173 Norwegian farms, Campylobacter status from 5568 flocks, and 44 explanatory variables were included. A generalized linear model with a logit link function was applied and analyzed using a stepwise elimination procedure. The challenge of analyzing the full model, including all variables with many parameter levels, was overcome by collapsing levels wherever meaningful, and by excluding variables with the highest number of missing values in the first steps of the stepwise elimination. An increased risk was associated with country; ie. Danish broiler flocks were more frequently colonized by Campylobacter than Norwegian flocks. Furthermore, the age of the broiler house, density of birds, level of biosecurity, length of downtime and type of drinkers were all found to be associated with the risk of the broiler flocks becoming colonized by with Campylobacter. The next step will be to extend the model to include data from the Netherlands, Poland, Spain, and the UK, as well as climate factors, to further investigate differences among different countries and different climatic regions of the EU.

P218. The multifactorial nature of the aetiology of human campylobacteriosis in Scotland.

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Human campylobacteriosis has increased by 79% in Scotland between 1990 and 2000, fell by 29% to 2005 and increased by 33% to 2011. Similar patterns were observed elsewhere in the UK. Differences in both age distribution (during the period 1990–2011 the incidence in young children fell by approximately 40% whilst there was a >300% increase in the elderly) and demography were observed during this period and the pattern of human disease did not simply correspond to differences in consumption of chicken meat. Here we combine results from empirical epidemiology, case-case, case-control, time series analysis and microbial sub-typing (source attribution, diversity and genetic distance) to unravel the changing factors that lead to the emerging trends of human campylobacteriosis. Risk factors associated with human disease (including proportion of cases that are rural, chicken consumption rates, foreign travel rates, proportion of population using proton pump inhibitors, frequency of genotype and attribution to source) change over time and vary by age. In addition in the animal reservoirs the prevalence and genotype also change over time. Only by combining these multiple factors is it possible to obtain a putative explanation for the changes in human disease over the last 25 years and the potential to make an informed view of how incidence rates may change in the future.

P219. How sporadic are campylobacteriosis cases? Results from a 5 year enhanced surveillance project in southwestern Alberta, Canada.

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Campylobacteriosis primarily incited by *C. jejuni* (CJ) is the most prevalent bacterial enteritis in Canada. Despite the high burden of illness associated with CJ infection, most cases are thought to be sporadic, posing a significant challenge to effective mitigation strategies. Southwestern Alberta (SWA) possesses a high prevalence of campylobacteriosis, with rates that are among the highest in North America. The region possesses an ≈50:50 urban-rural split, contains a single predominant watershed and a diverse agroecosystem that includes high densities of livestock. In an effort to examine factors underlying the high incidence of campylobacteriosis in SWA, we initiated a project to examine the molecular epidemiology of CJ affecting humans in the region (model ecosystem approach). CJ isolates (n=1350) recovered from stools submitted to the single centralized diagnostic facility serving the region from 2007–2012 were subjected to high-resolution genotyping using Comparative Genomic Fingerprinting (CGF). Genotypic data was compared to that from non-human CJ sources (≈4K concurrent from SWA; ≈10K from across Canada). The temporal distribution of CGF subtypes indicated that ≈33% of clinical cases in SWA comprised clusters (n=112) ranging in size from 2 to 13 cases. CJ subtypes in recurring case clusters indicated a strong association with cattle. Our results suggest that a significant proportion of cases in SWA are not sporadic, with cattle contributing to the epidemiology of campylobacteriosis. Currently, we are expanding the size of the genotyped CJ isolate collection from SWA (20K isolate target), and collating genotypic data with notifiable disease interview and whole genome sequence data.

P220. Freezing as an intervention strategy to reduce Campylobacter numbers isolated from chicken livers

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Aims: to determine the prevalence and numbers of campylobacters in samples of fresh and frozen livers purchased at retail across the UK. In addition, the freezing of chicken livers contaminated with *Campylobacter* was assessed to determine any potential for decontamination. Methods: Chicken livers naturally contaminated with campylobacters were subjected to freezing treatments to –15°C and –25°C for one day and seven days. Numbers of campylobacters on the livers were determined immediately before and after a 24 h or 7 d freeze treatment and daily during a three days post-thaw refrigerated storage. Major findings: Freezing for 24 h at –25°C caused reductions to campylobacters of up to 2 logs. Two freeze treatments each

for 24 h duration at –25°C, with refrigerated thaw in-between, caused reductions to campylobacters numbers of up to 3 logs. There were significant reductions to campylobacters numbers between the first and second freeze treatments. Livers sampled at retail contained up to log 4 cfu/g of liver. Frozen livers contained significantly lower numbers of campylobacters. Main Conclusion: Freezing chicken livers can reduce, but not eliminate, campylobacters. Impact: Were poultry processors to freeze livers destined for human consumption as part of routine processing, there is potential for a reduction in the foodborne illness associated with the consumption of imperfectly cooked chicken livers and processed derivatives, such as pate. This study provided evidence that reductions of more than one log cycle (about 90%) can be achieved for campylobacter numbers on livers if the organs are frozen and thawed before cooking.

P221. *Campylobacter jejuni* lineage and isolate source influence chicken colonisation: evidence for chicken specialisation

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Campylobacter jejuni is the leading cause of gastroenteric bacterial infectious disease in the industrialised world. As more *C. jejuni* strains are typed or have their genomes sequenced it becomes apparent that whilst some clonal complexes (CC) contain strains isolated from a range of sources including poultry, ruminants and wild birds (host generalists), others are more specific, containing a majority of strains isolated from only one of those host groups (host specialists). The basis of these varying ecological strategies is unknown, as is the link between epidemiological observations and *in vivo* phenotypes. More specifically, the possibility that host specialism is caused by a reduction in the ability to colonise other types of host species remains unclear. This work aimed to determine whether chicken specialist strains were able to colonise chickens more effectively than host generalists. Batches of 21-day-old broiler chicks were infected for seven days with two different tagged strains of *C. jejuni* from host generalist or specialist background. In both cases a strain from a chicken specialist CC was observed to colonise the birds to significantly higher levels than a strain from a host generalist CC originally isolated from chicken. These results suggest that chicken specialist strains colonise chickens better than strains from a host generalist CC.

P222. Killing *Campylobacter* using Hydrostatic High Pressure Treatment: efficient parameters and subsequent damages on the bacterial cells

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Campylobacter jejuni is one of the most intriguing human foodborne bacterial pathogen. Living in the animal guts and particularly in avian intestine as a commensal bacterium, this microorganism is frequently isolated from poultry meat products. Ultra high pressure (HP) is a promising alternative to thermal technology for microbial safety of foodstuffs with less organoleptic and nutritional alterations. Its application could be extended to meat products potentially contaminated by *C. jejuni*. Consequently, the influence of environmental (temperature and pH) and biological (strain) parameters on the inactivation of *C. jejuni* by HP was investigated. The results indicated an efficient pressure for a 7-log reduction of *C. jejuni* at 400 MPa with a possible reduction of the efficient pressure according to temperature, pH and strains. To evaluate the response of *C. jejuni* to this technological stress and subsequent recovery at a molecular level, a dynamic 2-DE-based proteomic approach has been implemented. After cultivation, *C. jejuni* cells were conditioned in a high-pressure chamber and transferred to fresh medium for recovery. The protein abundance dynamics at the proteome scale were analyzed by 2-DE during a full cycle of HP injury and subsequent cell recovery. Proteomic dynamics during this process sequence was explored from 69 spots showing statistical changes through time. Monitoring protein abundance through time unravelled the basic metabolisms involved in this cellular process and revealed that HP might eradicate cells from an oxidative burst which damaged cell membrane integrity.

P223. Transmission of *Helicobacter pylori*: the case of a multi-racial metropolitan

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Introduction and Aims: *Helicobacter pylori* are the major etiological agent of chronic gastritis and peptic ulcer disease, and are a risk factor for gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Thirty years after its first successful isolation, the actual mode of transmission of this bacterium remains a mystery. In this preliminary study, we aimed to study the diversity of *H. pylori* isolated from the three representative racial groups (Chinese, Malay and Indian) in Kuala Lumpur and postulated on the mode transmission in the multi-racial society. Methods: Representative *H. pylori* strains matched for disease background were selected for de novo sequencing on the Illumina HiSeq plateform. Major Findings: Phylogenetic analysis revealed diversity among *H. pylori* strains from this region. Interestingly, despite the three racial groups living in close proximity sharing food and water source, analysis of highly conserved genes among *H. pylori* strains yielded three distinct bacterial populations with distinct racial distribution. Main Conclusions: Highly diverse human and *H. pylori* population found in Kuala Lumpur provided a living model to investigate on the co-evolution of human and *H. pylori*, as well as testing different model of transmission. Data presented in this study defies the hypothesis that environmental sources may serves as a reservoir in the transmission of *H. pylori*; instead strongly supported an intra-familial transmission model. Impact of the Research: Preliminary data from this study provided important evidence to aid in establishing the mode of *H. pylori* transmission.

P224. Human gastric microbiome and molecular crosstalk with *Helicobacter pylori*

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Intro: More than half of the population worldwide are colonised with *H. pylori* but of these only minority developed symptoms. Apart from *H. pylori*, other microbiota species have also been related to gastritis. The aim of this work was to characterise the molecular crosstalk between *H. pylori* and other bacterial species in the gastric microbiome. Methods: Gastric biopsies were collected from symptomatic patients presenting at the University of Malaya Medical Centre (UMMC) Endoscopy Unit. Bacteria present in the samples were pre-enriched in brain-heart infusion broth prior to culturing on selective and non-selective media. Bacteria colonies were identified by morphological, biochemical and *16S rRNA*-based sequencing. Culturability of *H. pylori*, *Streptococcus mitis* and *Lactobacillus fermentus* in a multispecies condition was investigated *in vitro* using a cell culture insert fitted with a 0.4 mm polyethylene terephathalate membrane. Results: Non-*H. pylori* bacteria isolated from gastric tissue, includes *Bacillus* sp., *Klebsiella* sp., *S. mitis*, *Streptococcus* sp. of oral origin, *Neisseria mucosa* strain, *Burkholderia* sp., *Burkholderia fungorum*, *Burkholderia tuberum*, *Neisseria sicca*, *Micrococcus* sp., *Paenibacillus* sp. and *Vibrio* sp. Impact of research: These findings suggest that the human gastric microbiome is complex. In addition, co-culturing of *H. pylori* and *S. mitis* demonstrated possible molecular crosstalk between these organisms. Keywords: *H. pylori*, *S. mitis, L. fermentum*

P225. *Arcobacter thereius* Isolations from Human Stool: Another *Arcobacter* Queuing for a Role as Zoonotic Pathogen?

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Introduction: The genus *Arcobacter* contains 17 species of which three: *A.butzleri, A. cryaerophilus* and *A.skirrowii*, have been associated with human disease. *Arcobacter species* have been isolated from drinking water, feces from livestock and foods from animal origin but the knowledge about transmission, colonization in the human gut, pathogenicity and virulence remains limited. Methods: We used a protocol with inoculation of stool into a selective broth followed by solid selective *Arcobacter* media incubated in microaerobicconditions at a low temperature of 28ºC+-2ºC. Plates were screened after

72 hours by dark field microscopy for suspect, typical bluish colonies. Identification of isolates was performed by species specific m-PCR and confirmed by both phenotypic tests and Amplification Fragment Length Polymorphism analysis. Results: *A. thereius* was isolated from the stools of two patients with clinically confirmed enterocolitis. The first patient was a 3 year old toddler with recurrent diarrhea and failure to thrive. The second patient was a young woman of 29 years old with a flare up of Crohn's disease. Stool culture of both patients yielded no other bacterial pathogens. Discussion: These isolations again indicate a possible association with human disease of a not previously described *Arcobacter sp*. Both patients received symptomatic treatment and recovered without initiation of specific antibiotic therapy. Given the abundant presence of different *Arcobacter spp* in the environment, drinking water and livestock, the search for arcobacters and their zoonotic potential in humans should be intensified.

P226. Screening studies on feed additives in broiler *Campylobacter* dose response susceptibility model

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Feed additives might prevent *Campylobacter* colonization in broilers. To evaluate the efficacy of feed additives, an *in vivo Campylobacter jejuni* susceptibility model was validated, and two screening studies were performed. Each study consisted of 224 individually housed broilers (Ross308) that were fed drug-free mash diet supplemented with 8 single component feed additives, including MCFAs, MCFA-esters, essential oils, probiotics, or insoluble fibre, from day 11. Birds were inoculated with a mixture of 2 *C. jejuni* strains (ST45 and ST2324) at 21 of age, and sampled 5 days later. Eighty non-treated control birds were inoculated with 3.0, 3.5, 4.0 or 5.0 ^{10}Log CFU/bird. Each dietary treatment was represented by 16 birds, inoculated with 4.0 or 5.0 ^{10}Log CFU/bird. Data were analysed by logistic regression (SAS 9.3). Dose response relation in control birds were very similar in validation and screening studies, with inoculation dosis resulting in half of the broilers colonized ($CD_{50\%}$) ranging from 3.3 to 3.6 ^{10}Log CFU/bird ($P>0.5$). Simulations indicated a ±80% statistical power to detect shifts in $CD_{50\%}$ equal to +1.5 ^{10}Log CFU/bird. Reduced susceptibility ($P<0.05$) was observed for Heptanoic Acid (C7, 0.5%) and Caprylic Acid (C8, 1%), both increasing $CD_{50\%}$ with +1.0 ^{10}Log CFU/bird. Capric acid (C10, 1%) and Trans-cinnamaldehyde (0.04%) increased $CD_{50\%}$ with +0.9 ^{10}Log CFU/bird. Other treatments did not show an effect on *Campylobacter* susceptibility (P values > 0.20). Within this susceptibility model MCFAs were most consistent in reducing *Campylobacter* susceptibility, and consequently could be considered for prevention of *Campylobacter* colonization in broilers.

P227. Risk factors for *Campylobacter* colonisation in broilers depend on *Campylobacter* genotype

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To identify risk factors that contribute to *Campylobacter* colonization, sixteen ceca from broilers housed on hundred Spanish farms were cultured weekly. Farmers were interviewed related to farm characteristics, biosecurity measures, water supply, workers, buildings, cleaning procedures, litter use, and ventilation. Weekly additional information (hygiene, treatments) was collected by technicians. The most dominant species was *C. jejuni*, accounting for 74% of positive flocks. RAPD genotyping indicated 46% of these isolates were showing clonal appearance. *C. coli* accounted for 26% of colonized flocks. Three *Campylobacter* categories were defined and survival analyses were performed per category. All categories showed increased risk for colonization (RFC) when boot disinfection system was lacking at weekly check (Hazard Ratio (HR): 1.8–1.9, $P<0.1$). For *C. jejuni* isolates with clonal appearance, RFC was increased dramatically when chicks were brooded in limited part of the house (Hazard Ratio (HR) 13.5, $P>0.001$), with other poultry farms present within a 1 km range (HR 2.82, $P<0.01$), with farm animals present at the farm (HR 3.02, $P<0.05$), and when employees also worked on other farms (HR 2.05, $P<0.05$). For non-clonal *C. jejuni*, employees working on other farms was protective (HR 0.44, $P<0.05$), as were concrete floors surrounding the broiler house (HR 0.33, $P<0.01$). Furthermore, recent application of antibiotics reduced the RFC (HR 0.16, $P<0.05$). Contrary, for *C. coli* use of antibiotic increased RFC (HR 2.35, $P<0.05$). In conclusion, when studying risk factors for *Campylobacter* colonization, it is highly recommendable to consider genotype of isolates, as distinct epidemiology might exist.

P228. Insights into the Epidemiology of *Campylobacter* from Cape Town, South Africa

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Advances in bacterial typing have improved our knowledge of *Campylobacter* epidemiology; however, our understanding of the epidemiology of *Campylobacter* in developing countries remains limited. The aim of this study was to use whole-genome sequencing (WGS) to characterise *Campylobacter* from Cape Town, South Africa, a developing country. Draft genomes were assembled *de novo* for 40 clinical *Campylobacter* isolated from four public hospitals in Cape Town between November 2011 and March 2012. WGS was also carried out on 11 clinical *Campylobacter* collected from Red Cross War Memorial Children's Hospital between 1982 and 1989, which had been preserved as freeze-dried isolates. The resulting draft genomes were annotated using Bacterial Isolate Genome Database (BIGSdb) software (http://pubmlst.org/software/database/bigsdb/), and phylogenetic analyses were carried out with SplitsTree4. Both isolate collections included representatives of *Campylobacter jejuni* subsp. *jejuni*, *Campylobacter jejuni* subsp. *doylei*, *Campylobacter coli* and *Campylobacter upsaliensis*, with a high degree of genetic diversity within each species. Multilocus sequence typing data extracted from the genome sequences indicated that for *C. jejuni* subsp. *jejuni* and *C. coli*, Cape Town isolates largely corresponded to globally described disease-causing genotypes. In contrast, the majority of *C. upsaliensis* isolates corresponded to a lineage previously described as being restricted to South Africa. For all species, a number of the same lineages were identified in both collections. These data suggest that the epidemiology of *Campylobacter* from Cape Town, South Africa, is similar to, yet distinct from, that observed in high-income countries, and has likely been so since at least the 1980s.

P229. Spatial visualisation and exploration of *Campylobacter*-positive broiler farms in Great Britain

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Introduction: This study aimed to investigate the spatial distribution of *Campylobacter*-colonised broiler farms as well as the geographical distribution of particular MLST clonal complexes in Great Britain in order to generate hypotheses on explanatory factors that may be associated with the colonisation of broiler farms. Methods: Data on the *Campylobacter* status of 528 farms sampled at slaughter between 2007 and 2009 were included in the study. Spatial visualisation and exploration using the spatial scan statistic were conducted to identify significant local clusters of *Campylobacter*-positive farms. Results: The scan statistic identified areas where there was a higher risk for a broiler farm testing positive for *Campylobacter*. Local areas with higher frequency of farms colonised with particular MLST clonal complexes were also found. The identification of geographical areas with higher risk for *Campylobacter* colonisation in broilers could indicate the presence of risk factors related to geographical regional differences, such as landscape and climate or risk factors that might be linked with 'local' sources or practices. The predominance of varying MLST types in different areas could therefore be associated with different origins of infection, transmission pathways and exposure routes. Impact: Further studies on risk factors for *Campylobacter* colonisation related to geography and climate and the identification of molecular type-specific risk factors will help to improve the understanding of *Campylobacter* epidemiology in broiler flocks and facilitate the identification of more well targeted and efficient control programmes.

P230. Development of a q-PCR strategy to detect Campylobacter concisus within biopsy samples

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Aims: The gut microbiota plays a key part in Inflammatory Bowel Disease (IBD) pathogenesis. Certain bacteria are more prevalent in IBD patients and recently Campylobacter species, especially Campylobacter concisus, has generated much

interest as a potential causative organism. As all IBD Campylobacter work to date has used qualitative techniques, this study was initiated to develop a quantitative PCR strategy to quantify C. concisus abundance in human biopsy samples. Methods: Several Campylobacter genus and Campylobacter concisus specific primers were identified from the literature or designed and their optimum efficiency for q-PCR assessed. Once optimised, specificity checks were undertaken to ensure amplification only of intended bacterial species. Assessment of Campylobacter abundance was then performed on a selection of colonic mucosal biopsies. Major Findings: Following assessment of the various primer pairs, optimum primer pair combinations for Campylobacter genus amplification and for Campylobacter concisus amplification were defined with 90% and 92% efficiency respectively. These primer pairs were also shown to be specific to their target DNA. Analysis of biopsy samples indicated that levels of Campylobacter abundance were generally low with positivity only seen in spiked samples or following 2 rounds of PCR amplification. Conclusion and Impact of Research: We have developed a robust and effective q-PCR strategy to assess the abundance of Campylobacter species and also specifically Campylobacter concisus species. Although the levels of Campylobacter within the biopsy samples was below the detection limit of the assay, the approach can now be broadened to assess other sample types where Campylobacter organisms are present.

P231. Determination of antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from slaughtered chickens in Poland

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Poultry is a main reservoir and source of human *Campylobacter* infection. The aim of the present study was to test the current status of antibiotic resistance of *C. jejuni* and *C. coli* isolated from caeca and carcasses of slaughtered broilers in Poland. Minimum inhibitory concentrations (MIC) with the microbroth dilution method against 7 antimicrobials was carried out for 139 *C. jejuni* (55 from caeca and 84 from carcasses) and 115 *C. coli* (46 from caeca and 69 from carcasses). The prevalence of resistance among *C. jejuni* and *C. coli* isolates were as follows, respectively: nalidixic acid (81.3%, 80.0%), ciprofloxacin (81.3%, 80.0%), tetracycline (54.0%, 54.0%), streptomycin (19.4%, 48.7%), gentamicin (0%, 4.3%), erythromycin (0%, 1.7%), and chloramphenicol (0%, 0%). The majority of the *Campylobacter* isolates resistant to streptomycin, gentamicin, and tetracycline had the MIC values >16 mg/l. Moreover, 2 *C. coli* resistant to erythromycin had the MIC values >32 mg/l. Almost all strains resistant to nalidixic acid and ciprofloxacin, irrespective of the bacterial species, had the MIC values ≥64 and 4 mg/l, respectively. Resistance to antimicrobial agents was significantly more frequent in *C. coli* isolates compared to *C. jejuni* only for streptomycin ($p<0.05$), whereas such differences were not observed for the remaining antibiotics as well as between strains isolated from caeca and carcasses. In conclusion, a relatively high rates of antimicrobials resistance were found in *Campylobacter* isolated from Polish broilers. It should be issues of concern in public health because these antibiotics are frequently used in the treatment of human *Campylobacter* infections.

P232. Prevalence of multidrug resistant *Campylobacter jejuni* and *Campylobacter coli* from slaughtered broilers in Poland

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The objective of this study was to determine the occurrence of multidrug resistant *Campylobacter* spp. isolated from chicken in Poland. The strains were recovered from poultry caeca (55 *C. jejuni* and 46 *C. coli*) and carcasses (84 *C. jejuni* and 69 *C. coli*) at the slaughterhouse level. The isolates were investigated for antimicrobial susceptibility against 7 antibiotics belonging to 5 different classes: quinolones (nalidixic acid and ciprofloxacin), tetracyclines (tetracycline), aminoglycosides (streptomycin and gentamicin), macrolides (erythromycin), and amphenicols (chloramphenicol) by using a microbroth dilution method. Multidrug resistance, defined as resistance to more than two different classes of antibiotics, were detected in 66 out of 254 (26.0%) *Campylobacter* examined. *C. coli* were more often found as multidrug resistant since a total of 35.7% (41 out of 115) isolates showed multiresistance compared to 18.0% (25 out of 139) *C. jejuni* strains. The most common resistance profile revealed for both species was combination of ciprofloxacin-nalidixic acid-tetracycline, and streptomycin. Moreover, all (2 out of 254 *Campylobacter* examined) strains that displayed resistance to four different classes of antibiotics at the same time were identified as *C. coli* (one obtained from caeca and one from poultry carcasses) and shoved the resistance profile: ciprofloxacin-nalidixic acid-tetracycline-streptomycin-erythromycin. Thirty seven (14.6%) of

Campylobacter were susceptible to all antimicrobial agents tested. This study showed a high degree of multidrug resistance among *C. jejuni* and *C. coli* isolates from Polish poultry. Furthermore, the obtained results demonstrated that resistance of *Campylobacter* differs depending on the bacterial species.

P233. Genotyping, antimicrobial resistance and virulence markers patterns of the *Campylobacter jejuni* isolates from poultry in Poland

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Genetic variability, antimicrobial resistance (AMR) and virulence marker patterns of *C. jejuni* from poultry caeca (n = 55) and carcasses (n = 84) were investigated in this study. The isolates were typed by pulsed-field gel electrophoresis (PFGE) and examined for antimicrobial susceptibility against 7 antibiotics by using a microbroth dilution method as well as testing the prevalence 11 virulence markers by PCR. The genotypic relatedness of the isolates was determined by the analysis of the obtained profiles with the Bionumerics software. PFGE analysis with *Sma*I restriction enzyme of all 139 *Campylobacter* isolates resulted of 92 different PFGE types (with 95% similarity); among them 65 unique macrorestriction profiles were identified. The remaining 27 PFGE types appeared more than once and covered 18 types comprised of 2 *C. jejuni*, 4 profiles with 3 isolates, followed by 2 restriction types with 4 strains and 3 profiles with 5, 6 and 7 isolates, respectively. Moreover, 7 different AMR profiles and 10 various virulence markers patterns were displayed among *Campylobacter* tested. The analyzed *C. jejuni* population characterized a great genetic diversity and none of the PFGE types was found as predominant. Furthermore, in 18 cases the same PFGE types were identified in strains from caeca and corresponding carcasses which additionally possessed identical AMR and virulence marker patterns. Such findings suggest a cross contamination during the slaughter process. The matching PFGE, AMR and virulence markers profiles with the isolates from caeca and chicken carcasses confirming that prevention measures of *Campylobacter* in the poultry production are important.

P234. Comparison of molecular and antibiotic resistance patterns of *Campylobacter coli* isolated from slaughtered chickens in Poland

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This study was conducted to assess the correlation between antimicrobial resistance (AMR), virulence markers patterns and genotypes of *C. coli* isolated from poultry. A total of 115 *C. coli* strains from caeca (n = 46) and carcasses (n = 69) were examined for antimicrobial susceptibility (7 antimicrobials), and presence of virulence markers (11 genes) using PCR as well as pulsed-field gel electrophoresis (PFGE) with *Sma*I restriction enzyme. The cluster analysis was performed using Bionumerics software and isolates with >95% similarity were assigned to the same PFGE type. Altogether, 67 PFGE profiles, 11 virulence markers profiles as well as 10 AMR different patterns were found among 115 *C. coli* isolates examined. Furthermore, the analysis revealed 42 PFGE types detected only once and 25 different types common for 2 or more isolates as follows: 16 profiles common for 2, 5 types with 3 isolates, respectively, one profile with 4 strains and additional 2 types common for 5 isolates. The last 25th profile was the predominant and it covered 12 *C. coli* strains. Apart from this finding, 13 PFGE types were the same for the isolates from chicken caeca and carcasses obtained at the same time and from the same places. Furthermore, these pairs of strains demonstrated the same AMR and virulence marker profiles. The PFGE, AMR and virulence markers profiles of *C. coli* isolated from caeca and poultry carcasses showed the genetic diversity of this microbial group. The obtained results may also suggest a cross contamination of carcasses during the slaughter process.

P235. n3 Polyunsaturated Fatty Acid Diets Control *Campylobacter* in Broiler Chickens.

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The majority of cases of *Campylobacter* infections in humans are thought to be caused by the consumption of chicken. Contamination of carcasses can occur via two routes - from the spread of the bacterium from faeces to the carcass surface during processing or from infected muscle tissue and liver due to invasion of *Campylobacter* from the intestine . Invasion of

Campylobacter from the intestine is thought to be aided by inflammation; dietary n3 polyunsaturated fatty acids (PUFAs) are known to control inflammation which raises the possibility of their use to control *Campylobacter* infection in chickens. Broiler chickens were stocked at commercial densities and reared on diets containing either PUFAs or a control diet. Birds were infected with *C. jejuni* at 21 d and 10 birds euthanased at 7 d intervals post-infection. *Campylobacter* was detected in the liver using enrichment culture and in the caecum using direct plating onto mCCDA. Mortality, weight gain and leg problems were also measured. *Campylobacter* numbers in the caecum were significantly lower in birds fed diets containing PUFAs, as was the frequency of *Campylobacter*-positive livers compared to control diets. Body weight did not increase however, there were less mortalities and leg problems. We hypothesise that this is due to the n3 PUFA diets increasing bone strength and allowing heavy birds to remain mobile. Our data demonstrates that addition of PUFAs to the diet appears to reduce *Campylobacter* numbers in the intestine and the number of birds carrying it in the liver.

P236. A Role for Flies (Diptera) in the Transmission of *Campylobacter*?

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Infection of broiler flocks with *Campylobacter* spp. is difficult to prevent, especially during summer months when flock colonisation peaks. It has been hypothesised that the emergence of fly (Diptera) populations, their attraction to *Campylobacter*-contaminated animal faeces and their subsequent ingress into broiler houses may contribute to the transmission of *Campylobacter* spp. to broilers. To investigate this, *Campylobacter* spp. were cultured from flies collected from four broiler farms in North Wales and a dairy farm in Cheshire, England, from June to August 2012. In total, 1293 flies were cultured in 127 batches collected from the broiler farms and 1378 flies were cultured in 99 batches collected from the dairy farm. Four batches (3.15%) of flies from broiler farms and four batches (4.04%) from the dairy farm were positive for *C. jejuni*. The isolates were typed using multi-locus sequence typing (MLST), with STs belonging to clonal complex 45 (ST-25, ST-137, ST-1701) identified in flies caught on the broiler farms. On the dairy farm, isolates from two positive batches belonged to the bank vole associated strain ST-3704, with ST-270 also isolated. Malaise traps used to survey Diptera gaining access to broiler houses through ventilation inlets demonstrated a high diversity of flies on farms. Despite the low prevalence of *Campylobacter* spp. in flies found in this study, risk of transmission is potentially high as up to 612 flies were caught around broiler houses in a two hour period, representing multiple opportunities for flies to enter the house and infect the flock.

P237. Characterisation of Campylobacter species isolated from farm-caught Norway rats- a preliminary study.

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The role of contaminated chicken meat in the transmission of Campylobacter to humans is well documented; however there is no clear understanding of the origin of Campylobacter in chicken flocks. Norway rats (Rattus norvegicus) are common rodents in the farm environment and rats may play a role in introducing Campylobacter to chicken flocks. In this preliminary study, 35 Norway rats were caught from five livestock farms, and their intestinal contents or fresh faeces were analysed for the presence of Campylobacter. Positive Campylobacter spp. cultures were characterised using Multi Locus Sequence Typing (MLST) described by Dingle (2001). Six of 35 rats tested positive for Campylobacter spp. C. lari was isolated from one rat. The remaining five isolates were C. jejuni. MLST identified three unique C. jejuni STs, of which two were novel (ST 5129; ST 5130) and one had previously been identified in chicken, cattle and human stool samples (ST 586). Those novel STs displayed a combination of existing and new alleles. Analysis of the relationships between distinct alleles at each locus, using Unweighted Pair Group Method with Arithmetic Mean trees demonstrates that the novel STs consisted of alleles suggestive of widely diverse ecological niches, including both the farm and riparian environment. These results demonstrate that rats may be colonised by a wide range of Campylobacter types, some of which are usually ecologically separate, indicating they may provide a bridge between the riparian and farm environment. Further sampling of rats and whole genome sequencing of Campylobacter isolates is underway to explore this hypothesis further.

P238. Biofilm Building Capacity of Campylobacter and Salmonella Strains from the Poultry Production Chain

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Microbes persist attached to surfaces and not as pure cultures of planktonic growth in many natural habitats. Biofilms (BF) can be defined as an assembly of microbes being attached to a surface and furthermore being enclosed in a matrix of extracellular polymeric substances. BFs can be a reservoir for continuous bacterial contamination. A very important aspect of BFs in primary poultry production is their ability to harbor zoonotic agents like *Campylobacter* and *Salmonellae* posing a reservoir for continuous re-infection of poultry flocks. The Biofilm building capacity of different serotypes of *Salmonella enterica* derived from the poultry farm environment was investigated. In a second approach *Campylobacter* strains also originating from poultry production were investigated for their biofilm building capacity. Starting point for the investigation was the question whether farm-isolated *Salmonella* and *Campylobacter* strains with high importance for poultry meat and egg production are capable of forming a Biofilm under defined laboratory conditions. Biofilm building capacity was analyzed in a 96 well format. The Biofilm building capacity of a monospecies Biofilm was strongly dependent on the temperature used for incubation. In conclusion the Biofilm building capacity of poultry derived isolates is a function of adaptation to their host environment. Thus the control of Biofilm as a reservoir for *Salmonella* and *Campylobacter* in the farm environment is of crucial importance for the overall improvement of food safety.

P239. DGGE and qRT-PCR comparison of wild-type and chitosan "adapted" isolates of *Campylobacter jejuni* NCTC 11168.

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Intro: *Campylobacter jejuni is a notable burden to public health on a global scale. The biopolymer chitosan has shown various antimicrobial activities against both bacteria and fungi alike. With the ever increasing demand for more natural alternatives harbouring antimicrobial properties, as oppose to more chemically synthetic preservatives, chitosan has received attention with respect to use in food packaging and other food-related applications due to its GRAS nature.* Aim: *To determine differences between a wild-type and a chitosan "adapted" isolate of C. jejuni NCTC 11168 with respect to mutations and gene regulation using DGGE and qRT-PCR, respectively.* Methods: *DGGE analysis was performed using a DCode mutation detection system (BioRad, USA). A parallel gradient polyacrylamide gel (8%) was run for 3.5 hours at 200V after an initial 15 minutes at 20V. After staining the gel for 15 minutes, it was de-stained and visualised using a Gel Dock (G:Box, Syngene). Gene expression was analysed using qRT-PCR (StepOnePlus, Applied Biosystems) comparing chitosan- treated versus non-treated (control) for each isolate types in several genes of interest including CheY, FlaA and CmeB.* Results: *Preliminary studies suggest differences in gene expression in several genes when comparing the wild-type to the "adapted" isolate of C. jejuni when exposed to chitosan.* Conclusion and Impact of research: *Differences observed between the wild-type and chitosan "adapted" isolates highlight potential mechanisms whereby C. jejuni is able to overcome exposure to chitosan and thus remaining a problematic pathogen, despite its fastidious nature.*

Omics & Detection

P240. The role of Autoinducer 2 in *Campylobacter jejuni*

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Numerous bacteria communicate via a small interspecies-specific signalling molecule autoinducer-2 (AI-2) generated via LuxS. Many bacterial species use communication (Quorum sensing) modulating physiological functions and expression of virulence factors. However, little is known about AI-2 activity in *Campylobacter* species. In the literature the role of Quorum sensing in *C. jejuni* is discussed diversely. Analyses of Δ*luxS* mutant strains show various results. These are sometimes opposing results, which may be due to different experimental settings. For example temperature and growth phase seems to play an important role. Furthermore, the authors used different strains of *C. jejuni* Δ*luxS* mutant to confirm their studies. Additionally, most studies lack proof of complementing the Δ*luxS* mutant strains with AI-2 and/or a metabolic substance to confirm whether resulting phenotypes are due to metabolic function of LuxS or a consequence of disrupting cell communication. In this study we compare two Δ*luxS* mutant strains of *C. jejuni* in growth and motility at different temperatures (37°C, 42°C) in parallel. Furthermore we complemented each experiment with synthetic AI-2 or homocysteine as well as both combined. Moreover we examine the effect of AI-2 on *C. jejuni* wildtype and Δ*luxS* mutant gene expression. Our results show that addition of AI-2 induces a change in gene expression patterns, so we hypothesize that either a receptor for AI-2 might exist in *C. jejuni* respectively an active AI-2 import into the cell takes place. However, our data show that AI2 in *C. jejuni* Δ*luxS* mutant is not imported through an ABC transporter system.

P241. *Musca domestica* as a potential vector of *Campylobacter jejuni*

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The housefly (*Musca domstica*) is a well established mechanical vector of human pathogens including *Campylobacter* spp. that can cause infections of broiler chicken flocks, and through infected broiler meat can cause outbreaks of campylobacteriosis. We investigated how CFU of *Campylobacter* spp. changed in infected *M. domestica* larvae and pupae and whether *Campylobacter* spp. could be transferred between lifestages. Larvae that were kept at 25°C on a medium with *Campylobacter* spp. for four hours showed an initial CFU of 10^6 CFU/larvae, which subsequently dropped to 10^3 CFU/larvae only 8 hours after infection. Pupae originating from infected larvae showed initial CFU of 10^5 CFU/pupae. CFU stayed at this level over the next 8 hours and dropped after 24 hours to 10^4 CFU/pupae. We tested for expression in digestive and antimicrobial peptides (AMPs) between lifestages to see if this could explain the changes in CFU observed in larvae and pupae. CFU of *Campylobacter* spp. generally went down when expression of AMPs went up. *Campylobacter* spp. was transmitted between infected larvae to pupae, but not to the adult stage. The implications of these results on transmission under natural conditions will be discussed.

P242. Can propidium monoazide (PMA) be used for quantification of viable *Campylobacter* in chicken meat?

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Aims: To develop a rapid qPCR method to quantify *Campylobacter* in chicken meat, capable of distinguishing live *vs.* dead cells, as an alternative to the ISO 10272-2 microbiological culture method. Methods: Four different propidium monoazide (PMA) concentrations were evaluated on a live and dead *Campylobacter* range, artificially added to chicken rinse. To lower the limit of detection (LOD), different approaches were evaluated: decreasing the chicken rinse dilution, increasing the sample volume submitted to the DNA extraction and decreasing the volume of the DNA elution buffer, this using 3 different

DNA extraction kits. To further evaluate the PMA effect, different stresses of relevance to the chicken meat production chain, were induced to *Campylobacter* cells. A study comparing the PMA-qPCR and the ISO *Campylobacter* enumeration, using naturally contaminated chicken meat samples, was executed. Results: The PMA-qPCR method could be used to distinguish dead from live *Campylobacter* cells and allowed to enumerate the viable cells even when prior exposed to stress conditions. The PMA-qPCR method LOD was at 50cfu/g when the 1:5 rinse dilution, the NucleoSpin® Food kit using 2mL of sample submitted to DNA extraction and 100µL of DNA elution buffer were combined. A good correlation between both enumeration methods was achieved. Conclusion: The developed PMA-qPCR method was successfully used on chicken meat for *Campylobacter* quantification, even when the cells were under stressed conditions, at a LOD comparable to the ISO method. Research Impact: A real-time PCR method is now available to rapidly quantify viable *Campylobacter* during the chicken slaughter line.

P243. Use of a tetrazolium dye for monitoring growth, viability and biofilm formation of *Campylobacter jejuni* in food matrices

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Biofilms play an important role in infection strategies of bacterial pathogens, allowing survival and growth in hostile environments. Currently used detection methods for biofilm quantification often rely on dyes like crystal violet, which may often overestimate biofilm levels through non-specific staining. In this work we have developed a staining method that allows specific detection of metabolically active (viable) cells in biofilms, for use with the food-borne pathogen *Campylobacter jejuni*. Conversion of 2,3,5 triphenyltetrazolium chloride (TTC) to insoluble, red 1,3,5-triphenylformazan (TPF) was dependent on metabolic activity of *C. jejuni*. When used in food chain-relevant conditions by incubation with chicken juice (meat exudate), TTC staining allowed quantification of *C. jejuni* biofilm levels, whereas crystal violet staining resulted in high levels of non-specific staining of chicken juice components, regardless of the presence or absence of *C. jejuni* cells. Further investigation of the assay conditions lead to the development of an optimized assay for use with *C. jejuni*. Staining with TTC allows quantification metabolically active *C. jejuni*, and thus allows for quantification of viable cells in biofilms and food matrices. The TTC staining method can be adapted to quantify bacterial cell concentration in a food matrix model, where the accepted method of A_{600} measurement is not suitable due to interference by components of the food matrix. In conclusion, TTC staining is a low-cost technique suitable for use in biofilm analysis, allowing rapid and simple imaging of metabolically active cells and increasing the methods available for biofilm assessment and quantification.

P244. Rapid, easy and cost effective storage of *Campylobacter jejuni* cells and DNA

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Molecular typing of *Campylobacter jejuni* is an important tool for outbreak investigation, source attribution and molecular epidemiology. Conversion of the *Campylobacter* PCR-binary typing (P-BIT) system to a Multiplex Ligation-dependent Probe Amplification (MLPA) format, or MBiT, provides a rapid and cost effective molecular typing system for this important human pathogen. Current methods for the storage of *C. jejuni* cells and DNA are impractical for New Zealand routine testing laboratories meaning the majority of isolates are discarded without any sort of typing. In order to facilitate molecular typing of a greater percentage of *C. jejuni* isolates, rapid and cost effective methods for the short to medium term storage of cells and DNA are required. Seven methods for the storage of Chelex® extracted DNA were evaluated using three molecular methods (multiplex PCR, Multi-locus Sequence Typing [MLST] PCR and MBiT). MLST PCR was more sensitive to poor DNA quality resulting from less optimal DNA storage methods than the other two methods. DNAstable Plus (liquid) was the easiest to use and produced the best overall results. Survival of *C. jejuni* cells in a variety of media and supplements were evaluated. Viable cells were able to be recovered from sterile water with 7% horse blood stored at 4°C for at least 6 weeks. Rapid, easy and cost effective methods have been established for the short to medium term storage, and potentially transport, of *C. jejuni* cells and DNA, providing a practical means for greater numbers of isolates to be molecular typed using methods such as MBiT.

P245. PCR identification of polymorphic C. jejuni isolated from raw milk

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Campylobacter infections is one most common causes of gastroenteritis giving rise to approximately nine million of human Campylobacteriosis for year in EU. The most commonly isolated species from clinical infections is *Campylobacter jejuni* followed by *Campylobacter coli.* This species have similar biochemical reactions, not always sufficiently discriminating. The hippurate hydrolysis test is often used as the only phenotypic test capable of differentiating between the two species. Furthermore, all C. jejuni isolates possess the hippuricase gene (*hip0*) and almost all *C. jejuni* isolates express the hippuricase gene. For this reason many molecular identifications methods, like these based on the detection of the *hip0* gene, offer more reliable performance to differentiate *C. jejuni* from other *Campylobacter* species. However, even with these methods, some idendification difficulties can occurs in a minority of isolates . The presence of *C. jejuni* in bovine raw milk samples and fresh dairy products is increasing. In this study 6 *C. jejuni* strains isolated in bovine raw milk showed a polimorphysm of the *hip0* gene. In particular the sequencing of *hip0* fragments demonstrated a changing at sites 125 and 213 in which threonine (T) and valine (V) were respectively replaced with adenine (A) and isoleucine (I). According to the outcomes new primers for PCR identification of *C. jejuni* were designed and successfully implemented. The strains were further characterized by PFGE, MLST and microarray analyses.

P246. Exploring RNA targets of the translational regulator CsrA in *Campylobacter jejuni*

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Knowledge about post-transcriptional regulation including small RNAs (sRNAs) and RNA-binding proteins is still limited in the emerging food-borne pathogen *Campylobacter jejuni*. However, based on a comparative RNA-seq analysis of multiple *C. jejuni* strains, we have identified several conserved and strain-specific sRNAs, indicating that riboregulation contributes to gene regulation in *Campylobacter* and that there might be auxiliary protein factors involved. For example, *Campylobacter* encodes a homolog of the conserved RNA-binding protein, CsrA, which prevents translation by binding to GGA-motifs in the 5'UTR of mRNAs. Moreover, several sRNAs, CsrB/C, have been reported which can sequester and antagonize CsrA function. Deletion of *csrA* leads to pleiotropic effects and, e.g., affects oxidative stress response, biofilm formation and virulence in *C. jejuni*. However, its molecular mechanisms and RNA targets are still unknown in *Campylobacter*. Using a combination of co-immunoprecipitation and RNA-seq, we first catalogued the RNA-binding partners of CsrA in *C. jejuni*. Most of the highly enriched mRNAs belonged to genes involved in the flagellum and its assembly. However, we did not find any CsrB/CsrC-like sRNAs. Instead, most of the sequenced reads were mapped to *flaA* mRNA of the major flagellin. Moreover, CsrA did not only bind to 5'UTRs but in many cases also within the CDS, at mRNA 3'-ends or also between genes in polycistronic transcripts, hinting towards discoordinate operon regulation. Altogether, our data suggest that CsrA is involved in post-transcriptional regulation of multiple genes in *C. jejuni,* especially those involved in flagellum synthesis, and that it acts by novel modes of action.

P247. Degenerate CRISPR-Cas elements in *Campylobacter fetus*

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The loci containing clustered regularly interspaced short palindromic repeats (CRISPRs) and associated Cas genes, were characterized in *Campylobacter fetus* (*C. fetus*) subsp. *fetus* (Cff) and subsp. *venerealis* (Cfv). Both *C. fetus* subspecies are highly related at the genomic level but infect hosts and tissue with distinct preferences. Here we report a whole genome

sequencing (WGS) approach to identify the potential involvement of a CRISPR-Cas system in phage defense and the virulence of *C. fetus* subspecies. Roche 454 FLX Genome sequencing was performed for 21 *C. fetus* strains (4 Cff and 17 Cfv). Using Perl scripts, the contigs were assembled into a single scaffold. CRISPR sequences were identified using the CRISPRfinder (http://crispr.u-psud.fr) and WGS similarity searches using a local BLASTN tool. The locus in Cff strain 98v445 is inserted after a leucyl-tRNA and consists of two sets of CRISPR repeats flanking phage-like genes and six *cas* genes (*cas1* to *cas6*), similar to the CRISPR structure of Cff 82-40. This CRISPR type has not been found in epsilonproteobacteria and seems unique to *C. fetus*. In the same genomic location in strains Cff 04/554 and Cfv 97/608, only the CRISPR repeats and multiple inserted phage-like sequences were present. In 2 Cff and 16 Cfv, CRISPR sequences and phage-like insertions were found but no *cas* genes, that suggests the recombinational loss of *cas* genes. The degenerated CRISPR system in both subspecies and the phage-like insertions make it questionable if the CRISPR-Cas element in *C. fetus* has the function of an adaptive immune system.

P248. Investigating the presence of putative virulence genes in *Campylobacter concisus* genome of oral and clinical isolates

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In recent decades, studies have shown an emergence of fastidious *Campylobacter* species such as *C. concisus,* a coloniser of the human oral cavity, as potential causes of human enteric infections. *C. concisus* has been grouped into two genomospecies (A&B) based on the amplification of the 23S rDNA. The genetic diversity of *C. concisus* as a species was ratified after sequencing of *C. concisus* UNSWCD revealing that the number of genes in this strain was not equivalent to the previously sequenced genome of *C. concisus* 13826. This study investigates the presence of several putative virulence genes in *C. concisus* genome including *cjaA* (*Campylobacter jejuni* antigen A, CCC13826_0664), *Dnaj* (heat-shock protein, CCC13826_0965), *zoT* (zonula occludens toxin, CCC13826_2276), *flaB* (Flagellin B, CCC13826_2297) and *flaC* (Flagellin-like protein FlaC, CCC13826_2187). Genomic DNA was extracted from 31 *C. concisus* isolates (15 clinical and 16 oral) and three other *Campylobacter* spp. PCR was then performed with oligonucleotides designed to target these nominated genes. Our findings suggest that the *dnaJ, flab, cjaA* and *zoT* genes unlike the *flaC* gene might not exist in some isolates or could have different nucleotide sequences compared to *C. concisus* 13826 genome sequence. These findings could improve our knowledge and provide a deeper insight in the association of *C. concisus* with human disease. Further studies are underway to evaluate the virulence characteristics of clinical and oral isolates from different genomospecies to assess the role of these genes in the pathogenesis of this bacterium.

P249. Spirality and genetic variability of *Campylobacter jejuni*

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Spiral shape is relatively rare in the bacterial world. Most bacteria are described as either coccoid or rod, with only 13 prokaryotic genera known to include any spiral or vibroid species. Studies of the spiral-shaped pathogens *Camyplobacter* and *Helicobacter* demonstrate that in addition to being an interesting and poorly understood cell shape, spirality has important roles in adhesion, invasion and host colonisation. To determine proteins involved in *C. jejuni* spirality, a random transposon mutant library was screened by light microscopy for differences in cell shape. The screen revealed spiral-, rod-, crescent- and coccoid-shaped mutants and the genetic causes for these morphological differences are under investigation. In addition, we are investigating the physical and phenotypical behaviours of a range of the different cell shape mutants. Whole genome sequencing of a selection of our transposon mutants has uncovered a number of SNPs in the genomes. This data supports the hypothesis of the genetic malleability of *C. jejuni* and emphasises the need for care when interpreting phenotypic observations of defined and random *C. jejuni* mutants.

P250. Interaction of Pathogenic Proteins from *Helicobacter pylori*

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Aims: *Helicobacter pylori*（*H. pylori*）, an infectious bacterium, is known to provoke atrophic gastritis and gastric carcinoma. The type IV secretion system expressed in *H. pylori* is responsible for abnormal immune response, alternation of gene regulation and cytoskeleton which are the primary pathogenic factor for gastric cancer. The other pathogenic proteins maybe has serious effect on the carcinogenic potency of *H. pylori* including secreting chaperones, urease, vaculating toxin and so on. Until now, molecular mechanism of these pathogenic proteins jointly participated into the pathogenic process is poorly understood. Methods: The interaction relationship among pathogenic proteins in *H. pylori* will be investigated in our research with the combination of yeast two-hybrid, tandem affinity purification technique and mass spectra. The pathogenic proteins interaction relationship will be studied in great detail and constructed with a combination of bioinformatics analysis. Major finding: An interaction occurring between a secreting chaperone and a key protein related with supplying energy was identified by yeast two-hybrid in this study. Main conclusion: This kind of interaction implied a cardinal significance for *H. pylori* colonization. Impact of research: On the basis of these findings, bio-macromolecular interaction mechanism and metabolic network of pathogenic proteins interaction taking part in regulation in vivo will be highlighted on. It is also hence of great theoretical and practical significance to unraveling the pathological state of the other infectious bacterium in pathogenic proteins interaction.

P251. Differential RNA-sequencing (dRNA-seq) of *Campylobacter fetus* subspecies.

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We used dRNA-seq to investigate the primary transcriptomes of *C. fetus* subspecies. The bovine isolate *C. fetus* subsp. *venerealis* 84-112 and the human isolate *C. fetus* subsp. *fetus* 82-40 were grown on blood agar plates and RNA was isolated to construct differential cDNA library pairs; one library from untreated total bacterial RNA and the other enriched for primary transcripts by terminator exonuclease treatment. Sequencing of cDNA libraries was performed on an Illumina HiSeq 2000 platform, which yielded about 29 million reads per strain on average. Of these, more than 91% could be mapped to the respective genomes. Annotation of transcriptional start sites (TSS) revealed 646 TSS on the leading strand and 574 TSS on the lagging strand of strain 82-40 and 1,457 TSS on the leading strand and 1,132 TSS on the lagging strand of strain 84-112, respectively. Classification of TSS according to their location relative to the surrounding open reading frames (*orfs*) revealed a variety of transcripts with TSS located upstream and internal to their respective *orf* but also included antisense transcripts. Analyses of consensus promoter sequences showed an extended Pribnow box (tgnTAtaAT) as the -10 motif in both subspecies and that the typical bacterial –35 motif is replaced by a periodic AT-rich signal upstream of position –14. This atypical structure is also known for other Campylobacterales (e.g. *Helicobacter pylori, C. jejuni*). Our dRNAseq approach is a first step towards a better understanding of gene regulation of the niche-adapted pathogen *C. fetus*.

P252. In search of persistence factors of *Campylobacter jejuni* in the chicken

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The intestinal pathogen *Campylobacter jejuni* persists in numerous different hosts and is frequently transmitted to humans. Chicken are considered to be the main source of *C. jejuni* infection. However, some *C. jejuni* strains, as ST-21, are generalists as they seem to be more efficient in colonizing different hosts. Possible fitness factors of *C. jejuni* contributing to this

phenotypic flexibility in changing environments and hosts are not yet identified. Additionally, it still remains unclear, whether and how genetic adaptation of the bacterium is taking place in any particular host or host individuum. To investigate the interaction of *C. jejuni* with its main host chicken *in vivo* and to study the genetic adaptation to chicken, we infected one chicken group with a human ST-21 *C. jejuni* strain and a second group with a chicken ST-21 *C. jejuni* strain. Using whole genome sequencing of the input strains and two reisolates genetic changes during the course of infection were analyzed. Gene expression analyses of chicken response to *C. jejuni in vivo* and *in vitro* as well as expression studies of numerous bacterial factors, which might be important for chicken colonization, in the chicken, were conducted. Taken together, the data obtained from the whole genome comparison provided evidence for genetic adaptation of *C. jejuni*, in particular through phase variation, *in vivo* within a short-term period of two weeks. Furthermore, the transcript analyses demonstrated that *C. jejuni* has an inflammatory effect in chickens although it is able to persist chronically in the avian host.

P253. Growth kinetics of *Campylobacter* and ESBLs in selective enrichment broths

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According to the ISO-protocol for detection of thermotolerant *Campylobacter* spp. in food and animal feeding stuffs (ISO 10272-1, 2006), Bolton broth (BB) is used which is mixed 10:1 with the food sample. After enrichment, campylobacters are isolated on mCCDA, which is often complicated, since abundantly growing Extended Spectrum Beta-Lactamase producing bacteria (ESBLs) make it hard to recognize and isolate *Campylobacter* colonies. Other enrichment broths, such as Preston broth (PB) and BB plus clavulanic acid (BBc) have been suggested. However, detailed growth dynamics of *Campylobacter* and its competitors during enrichment remain unclear, where these would provide a solid base for further improvement of the enrichment procedure of *Campylobacter*. With a revision of the ISO-10272-protocol at hand, we measured the growth kinetics in PB, BB and BBc of several strains of *C. jejuni and* ESBLs separately and in combination. Also, growth dynamics were evaluated during enrichment of naturally contaminated chicken samples. No significant difference in growth kinetics were found using a pre-enrichment step of 4 h at 37°C compared to immediate enrichment at 41.5°C. The yields and often the growth rates of *Campylobacter* in co-culture with ESBLs were lower than in pure cultures, indicating severe suppression of *Campylobacter* by ESBLs. PB and BBc successfully inhibited growth of ESBLs and are therefore a better choice as enrichment medium for possibly ESBL-contaminated samples. Therefore, the choice in the revised ISO-10272-protocol for use of PB in samples where high levels of background flora is suspected, is well supported by these experiments.

P254. Finalisation of the revision of International Standard ISO-10272 for detection and enumeration of *Campylobacter* in food and animal feed.

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The updated (March 2013) revision of ISO 10272 is now ready for final technical and editorial international voting. **Parts 1A, 1B, and 1C** for **detection by enrichment**, depending on expected numbers of campylobacters and the level of background microflora. Part 1A: Bolton broth (4–6 h at 37°C then 40–48 h at 41,5°C), isolation on mCCDA and a second selective medium, with a principle different from mCCDA. E.g. cooked or frozen products (except frozen raw poultry meat). Part 1B: Preston broth (22–26 h at 41,5°C) plus isolation on mCCDA. E.g. raw meats, raw milk, frozen raw poultry meat. Part 1C: Direct isolation from sample material or a primary dilution onto mCCDA. E.g. faeces, poultry caecal contents or raw poultry meat. Like for agar plates, all enrichment broths to be incubated in micro-aerobic atmosphere. **Part 2**: plating on mCCDA for **enumeration** (in single, ISO 7218:2007). **Part 3**: **semi-quantitative estimation** of the *Campylobacter* level based on qualitative detection in a single range of ten-fold dilutions of a sample in Bolton broth. ISO/TS 10272-3 is currently under international voting for continuation/revision or withdrawal. Originally, analysis of **samples from primary production** was to be described in a separate **part 4**, but the enlargement of the general scope enabled these type of samples to be taken up into parts 1B and/or 1C. **Confirmation in all parts** are harmonised to include microscopic examination, detection of oxidase and absence of aerobic growth at 25°C. Optionally, *Campylobacter* species are identified by specific biochemical tests (catalase, hippurate hydrolysis, indoxyl acetate).

P255. Validation of the revised ISO 10272 for detection and enumeration of *Campylobacter* in food and animal feed under EU Mandate M/381.

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The European Commission Mandate M/381 asks the European Committee for Standardization (CEN) to provide standardized and validated methods related to food hygiene legislation. Fifteen analytical methods need to be fully validated by collaborative trials in which different laboratories participate to establish the performance characteristics of these methods. The revised ISO 10272 for detection (part 1) and enumeration (part 2) of *Campylobacter* in food and animal feed will also be validated under this mandate.

Table 1. Details on the validation studies.

	Detection ISO 10272-1	**Enumeration ISO 10272-2**
Project leader	Ida Jongenburger	Wilma Jacobs
Laboratory	NVWA, NL	RIVM, NL
Matrices studied	5	5
Sample size (except for caeca)	25 g	10 g
Inoculation levels	Blank	Blank
	Low, 5-10 cfu/sample	Low, eg 103 cfu/g
	-	Medium, eg 105 cfu/g
	High, 50–100 cfu/sample	High, eg 107 cfu/g
Number of samples per level	8	2
Total number of samples/lab	120	40
Number of participating labs	15	12
Number of valid data sets needed	10	8

Table 2. Matrices/strains for both the detection and enumeration studies.

Matrix	**Strain**	**Detection method**
Frozen minced meat (pork/beef)	*C. coli*	1-A (Bolton)
Lettuce	*C. jejuni*	1-A (Bolton)
Raw milk	*C. jejuni*	1-B (Preston)
Boiler skin	*C. coli*	1-B (Preston)
Broiler caecal material	*C. jejuni*	1-C (direct plating)

EU NRLs-*Campylobacter* are invited to participate in the validation. The enumeration study will take place in June, the detection study in October 2013. Results of the enumeration study will be presented at CHRO2013 in Aberdeen.

P256. "Novel *in vivo* expression systems for *Campylobacter* and *Helicobacter* species."

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A bottleneck during *Campylobacter* and *Helicobacter* experimental design is the paucity of available genetic tools. Common bacterial expression systems such as the *lac* promoter of E. coli, display reduced or no function in CHRO. We have addressed the need for effective, versatile *in vivo* gene expression systems for use in complementation, functional analysis, native protein production and reporter expression. Three native promoter/Shine-Dalgano regions from *C. jejuni* (*porA*), *H. pylori* (*ureI*), and *H. pullorum* (*flaA*) were used to construct suicide vectors with promoter-gene fusions and their subsequent integration onto the host chromosome. Promoter strengths were assessed in C. jejuni by fusion to a codon-optimised *gfp* gene and monitoring of whole cell fluorescence. All three constructs produced strongly fluorescent cells and importantly each displayed different promoter strength. Analogous constructs for the chromosomal expression of the host *hgpA* gene in *H. pullorum* displayed a similar expression pattern to those observed in *C. jejuni* as judged by Western blotting. Furthermore, the *porA* promoter-driven expression levels were sufficient for the tractable purification of milligram quantities of the HgpA protein. This new set of constructs allows constitutive recombinant gene expression from stable chromosomal insertions with a choice of expression level and will provide a useful resource for multiple applications.

P257. From Farm-to-Fork : Merck *Singlepath® Direct Campy Poultry* Rapid Test Kit for Direct Detection of *Campylobacter spp.* in Faecal and Caecal Samples from Live Chicken

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Aims: To provide a fast, reliable, simple detection method for *Campylobacter spp.* from faecal and caecal samples of high-shedding (>7.0 log10 CFU/g) broiler chickens, suitable for analysis both on farm and in the laboratory. *Methods:* A gold-labelled antibody sandwich Lateral Flow assay for *Campylobacter spp.* combined with a non-enrichment sample preparation protocol were developed to enable a time-to-result of within 1 hour from sampling. Field study evaluations both on-farm (faecal and caecal droppings) and at slaughterhouse (caecal contents) used a cross-seasonal representative set of broiler chicken faecal/caecal samples. Reference method comparison was ISO 10272 method and q-PCR. *Major Findings:* In an on-farm field trial of faecal droppings, *Singlepath® Direct Campy Poultry* achieved a sensitivity of 88.9% (% correctly classified positive) and a specificity of 91.0% (% correctly classified negative) based on a Limit of Detection of >6.0 $\log_{10}$ CFU/g of faeces. Overall agreement with q-PCR was 90.6%. In a slaughterhouse field trial of caecal contents, *Singlepath® Direct Campy Poultry* achieved a sensitivity of 92.4% (% correctly classified positive) and a specificity of 95.2% (% correctly classified negative) based on a Limit of Detection of >6.0 $\log_{10}$ CFU/g of faeces. Overall agreement with cultural plate count (ISO 10272) was 93.1%. *Main Conclusion and Impact of the Research:* Merck *Singlepath® Direct Campy Poultry* Rapid Test Kit provides an alternative, fast and simple method for detection of high shedding (>7.0 $\log_{10}$ CFU/g of faecal/caecal sample) *C.jejuni* and *C.coli* broiler chicken flocks, on-farm or at slaughter, and can assist in monitoring *Campylobacter spp* status of flocks and in slaughter scheduling.

P258. An enhanced technique combining pre-enrichment and passive filtration increases the isolation efficiency of *Campylobacter jejuni* and *Campylobacter coli* from water and animal fecal samples

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Aims: Improved isolation techniques from environmental water and animal samples are vital to understanding *Campylobacter* epidemiology. Methods: In this study, the efficiency of selective enrichment in Bolton Broth (BB) followed by plating on charcoal cefoperazone deoxycholate agar (CCDA) (conventional method) was compared with an approach combining BB enrichment and passive filtration (membrane method) adapted from a method previously developed for testing of broiler meat, in the isolation of thermophilic campylobacters from surface water and animal fecal samples. Major Findings: The conventional method led to recoveries of *Campylobacter* from 36.7% of the water samples and 78.0% of the fecal samples; similar numbers, 38.3% and 76.0%, respectively, were obtained with the membrane method. To investigate the genetic diversity of *Campylobacter jejuni* and *Campylobacter coli* obtained by these two methods, isolates were analyzed using Comparative Genomic Fingerprinting, a high-resolution subtyping technique. The conventional and membrane methods yielded similar numbers of *Campylobacter* subtypes from water (25 and 28, respectively) and fecal (15 and 17, respectively) samples. Main Conclusion: Although there was no significant difference in recovery rates between the conventional and membrane methods, a significant improvement in isolation efficiency was obtained by using the membrane method, with a false-positive rate of 1.6% compared with 30.7% obtained using the conventional method. Impact: In conclusion, although the two methods are comparable in sensitivity, the membrane method had higher specificity, making it a cost-effective procedure for the enhanced isolation of *C. jejuni* and *C. coli* from water and animal fecal samples.

P259. Whole genome analysis demonstrates that ST-21 complex *Campylobacter jejuni* isolated from chicken meat, slaughter calves, and human disease are indistinguishable.

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Genetic attribution studies based on seven locus multi-locus sequence typing (MLST) have indicated that a large proportion, potentially the majority, of human campylobacteriosis is derived from the consumption of retail chicken meat (Sheppard *et al.*, Clin Infect Dis. Apr 2009). These studies are, however, complicated by the fact that many isolates from human disease, chickens, cattle, and sheep belong to the same clonal complex, the ST-21 complex, which potentially results in misattribution. *Campylobacter jejuni* were obtained from human cases, animals, and food in Denmark in 2007–2008. 147 samples were ST-21, which constituted 13–17% of the isolates from domestic human cases, calves, broiler chickens, and Danish-produced chicken meat. WGS were obtained by Illumina sequencing and analysis within the pubMLST.org/campylobacter website. Comparisons of the genomes were undertaken using both 53 ribosomal MLST loci (Jolley *et al.*, Microbiology. Apr 2012) and a set of 1643 loci from ST-21 isolate NCTC11168 (Gundogdu *et al.*, BMC Genomics. Jun 2007). The analyses demonstrated appreciable diversity among the isolates, but no genealogical relationship of isolate with source, as essentially all genotypes were populated with samples from all sources. Additionally, the distribution in the Danish data set was indistinguishable from 102 ST-21 isolates collected from humans in Oxfordshire, UK, 2010–2012. Taken together, these data show that, at the WGS level, ST-21 isolates cannot be discriminated by source or geography. This is an important finding meaning that even at the endpoint resolution for sequence typing, source attribution within *C. jejuni* may not be possible for this common sequence type.

P260. Whole Genome Analysis of 'Campylobacter stanleyi' a novel hydrogen-dependent enteric pathogen isolated from humans.

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'Campylobacter stanleyi' is the name proposed for a novel hydrogen-dependent enteric pathogen detected in several cases of human gastroenteritis between 1997 and 2006. The bacterium was associated with relatively mild but persistent diarrhoea and patients with a link to the Indian subcontinent. The whole genome sequence of 'C. stanleyi' was determined using a Roche 454 Junior. Phylogenetic comparison with previously sequenced *Campylobacter* species was carried out using classical taxonomically useful genes such as 16S rRNA and the pglB gene. These analyses suggested that 'C. stanleyi' was clearly a distinct species that was most closely related to *C. avium* and *C. upsaliensis*. Whole genome sequencing facilitates the application of ribosomal multi locus sequence typing (rMLST) which is a powerful, tuneable tool for elucidating the phylogenetic relationship between bacteria. When the genome was examined with rMLST (based on 46 loci) it was evident that 'C. stanleyi' was much more divergent from the existing members of the *Campylobacter* genus than anticipated, forming a distinct branch quite separate from the clade that contained *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* and indeed many of the other *Campylobacter* species. The genome also contained several interesting features such as a fragmented N-linked glycosylation locus. Next Generation Sequencing allows the rapid generation of whole bacterial genome sequences, which facilitates the application of rMLST and other approaches that shed new light on the phylogenetic relationship between bacteria.

P261. Has MALDI-TOF MS the potential to replace 16S rRNA-gene-sequencing for identification of rarely isolated *Campylobacter* species (non-*Campylobacter jejuni/coli*)?

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Background: The 16S rRNA-gene-sequencing has become a valuable diagnostic tool for identification of slow growing, fastidious microorganisms with poor biochemical activity. MALDI-TOF MS has revolutionized microbiological diagnostics.

The purpose of this study was to evaluate the potential of MALDI-TOF for identification of *Campylobacter species* (*C.* spp.). Methods: A database search for non-fecal *C.* spp. isolates, isolated since 2004 at the Institute of Hygiene was done. Subsequently, from the 13 found *C.* spp., 6 isolates were re-cultured together with corresponding reference strains. All cultures were analyzed with MALDI-TOF after growth of 24h, 72h and 120h using the smear method for inoculation on the MALDI target together with HCCA Matrix. Analysis was performed with the MALDI-TOF-MS AXIMA™ Assurance (Shimadzu) and the database application SARAMIS™ (bioMérieux). Results: From 2004 to 2012, 13 *C.* spp. were isolated. Through 16S rRNA-gene-sequencing 6 different *C.* spp. (non-*Campylobacter jejuni/coli)* were identified. Using MALDI-TOF, from the 6 available clinical isolates, one *C. fetus* isolate and the corresponding reference strains were identified to the species level with a perfect score of 99.9%. It was not possible to identify the other isolates with MALDI-TOF neither to genus nor to species level. Conclusion: Although the majority of *C.* spp. isolated from clinical specimens are not integrated in the MALDI-TOF database so far, we expected at least the identification to genus level. Currently, MALDI-TOF has not the potential to replace 16S rRNA-gene-sequencing for identification of *C.* spp. However, an up-date of the MALDI-TOF MS database could probably solve this problem.

P262. Duplicated *tatA* genes in *Campylobacter jejuni*: Distinct roles in assembly of the electron transport chains

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Background and Aims: *Campylobacter jejuni* uses a complex set of electron transport chains to ensure growth with a variety of electron donors and alternative electron acceptors, some of which are known to be important for host colonisation. A significant number of the electron transport enzymes are localised in the periplasm by the twin arginine translocase (Tat), a secretion system that moves fully folded proteins across the cytoplasmic membrane. The Tat translocase normally consists of three proteins, TatA which forms the translocation pore and a TatBC complex, which recognises proteins containing the RR targeting motif in their signal sequences. Methods and Major Findings: We have discovered that *C. jejuni* encodes two distinct *tatA* genes and we have investigated the contribution of each gene to the correct localisation of a range of important redox enzymes to the periplasm. Single and double mutants were constructed in *tatA1, tatA2, tatA1tatA2*, *tatB, tatC* and *tatBC* genes. Assays for formate dehydrogenase, sulphite dehydrogenase, nitrate reductase, methylmenaquinol fumarate reductase (Mfr), TMAO reductase and the multicopper oxidase CueO were performed with intact cells or with periplasmic fractions. The results indicate distinct roles for each TatA paralogue, with TatA1 being the major player in the system. TatA2 seems to have a more specialised role relating to nitrate reductase localisation. Conclusions and Impact: The Tat system may be a target for intervention against *C. jejuni* colonisation are our data suggest that the mechanism of Tat translocation in this bacterium is more complex than previously realised.

P263. Comparative Genomic Fingerprinting: Leading towards enhanced surveillance of *C. jejuni* in BC

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Background: *Campylobacter jejuni* is one of the most common causes of human bacterial gastroenteritis worldwide. However, no rapid or cost-effective sub-typing tools are in routine use for surveillance of this organism. A novel multiplex PCR assay for the rapid and high resolution sub-typing of *Campylobacter jejuni*, called Comparative Genomic Fingerprinting (CGF),

was recently developed and evaluated by the Public Health Agency of Canada. Methods: The BC Public Health Microbiology and Reference Laboratory (BCPHMRL) previously initiated an evaluation of the CGF25 assay, for applicability to outbreak investigations, using clinical outbreak and sporadic isolates from BC. However, little is known about the distribution of clinical *C. jejuni* CGF subtypes in BC and Canada. CGF is therefore being applied prospectively for a subset of clinical *C. jejuni* isolates submitted to the BCPHMRL, in order to establish baseline distributions of current clinical CGF25 subtypes in BC. Results: All outbreak isolates linked via CGF25 were also confirmed via epidemiology and/or alternate sub-typing methods (MLST/PFGE). Whole genome sequencing and variant analysis was also performed on selected isolates from each outbreak. Dendrograms generated from variant analysis confirmed CGF25 results. The prospective study yielded a broad distribution of CGF25 subtypes in all isolates typed to-date. Conclusions and Impact: The CGF25 assay was demonstrated to be a useful and cost-effective tool for sub-typing outbreak isolates of *C. jejuni* at the BCPHMRL. This, in combination with a broad distribution of CGF25 subtypes, suggests the CGF assay will allow for effective surveillance of *Campylobacter jejuni* in BC and potentially worldwide.

P264. Completion and Analysis of the *Campylobacter peloridis* Genome and Methylome using Next-generation Sequencing.

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Campylobacter peloridis is a member of the *lari* subgroup of campylobacters. Strains of this species have been isolated from clinical samples and shellfish. As part of a project to sequence all taxa within the *Campylobacter* genus, the genome sequences of two *C. peloridis* strains (RM14092 = LMG 23910^T and RM2824 = LMG 11251) were determined. Draft genomes were constructed using Roche 454 FLX+ reads; however, it became apparent during closure that the highly repetitive nature of these two genomes prohibited closure by Roche/Illumina/Sanger sequencing alone. Therefore, to close and complete these two genomes, PacBio continuous long read (CLR) data was utilized. Together, these four sequencing methods were able to complete the *C. peloridis* genomes and high-depth Illumina HiSeq reads were used to characterize the *C. peloridis* hypervariable GC tracts. Moreover, the CLR data could be used to characterize the methylomes of the two strains. The two *C. peloridis* strains were approx. 1.7 mb in size with a %G+C of 28.5. Strain RM14092 contains an approx. 48 kb megaplasmid and a 3.6 kb cryptic plasmid. The gene content of the two *C. peloridis* strains is very similar to that of *C. lari lari* strain RM2100 and other members of the *lari* subgroup. The highly repetitive regions in strain RM14092 are confined primarily to two loci: a ~62 kb region comprised of genes of unknown function and a ~110 kb region associated tentatively with type VI secretion. Analysis and characterization of the two genomes with respect to related taxa will be discussed.

P265. Genome Content Analysis of a Group of Potentially Host Adapted *Campylobacter jejuni*

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Typically, *Campylobacter jejuni* is associated with poultry as the source, whereas *Campylobacter coli* is most frequently associated with food production mammals such as cattle and pigs. However, although human *C. jejuni* infection is most commonly associated with poultry (either through consumption of poorly cooked food, contact with raw meats, or direct contact with birds/faecal matter), it is accepted that poultry is not the sole source of *C. jejuni* infection in humans. Previous research identified a MLST Sequence-Type complex of *C. jejuni* (ST-Complex 403) with a strong bias towards pigs as source (89%) during a survey of abattoirs in the UK in 2000/2001. This same ST-complex has subsequently been associated with human cases of campylobacteriosis and in particular with a large number of isolates from the Dutch Antilles island of Curacao. The research presented here aims to identify any unique properties of isolates within this ST-complex which may explain this host association, or indicate host adaptation. Six isolates from ST-complex 403 were selected for sequencing, representing different sequence types within the complex. Following assembly and annotation, EDGAR was used to identify the core and pan genome for 33 C. jejuni and C. coli genomes; from which genes of interest present in or missing from ST-403 C. jejuni were discerned. Data presented will show selected genes of interest identified during comparative genome analysis that may give this organism an advantage in the porcine host and will also consider the extent.

P266. *Okadaella gastrococcus* is a novel Gram-negative *Streptococcaceae*

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Background: *Okadaella gastrococcus* (*Og*) is the name proposed for a Gram-negative coccoid gastric bacterium with fimbriae and unipolar flagella. They are facultative anaerobic, motile, urease, catalase, oxidase, PYR negative, and arginine aminopeptidase, H_2S positive and 0.2–0.75μm in diameter. *Og* co-exists with *Helicobacter pylori* in various gastropathies. Intracellular presence of *Og* has been demonstrated in the classic gastric carcinogenic cascades. Aim: to study the molecular analysis of *Og* and to propose a new classification. Methods: Data over 16 years were used for phenotypic studies. A DNA-DNA hybridization analysis (DDHA) of *Og* (ATCC BAA-2258, NBRC107862, GenBank HQ699465) and DNA G+C content analysis, the growth temperature, NaCl tolerance and pH studies over 5 days under anaerobic condition were performed by TechnoSuruga laboratory. *Streptococcus parasanguinis* (ATCC 15912) was used for DDHA. 16S rRNA gene sequencing analysis and the phylogenic tree reconstruction using the results were performed by Mitsui Norin. Results: Previously known phenotypic natures were confirmed. Growth was observed at 20–45 ℃, 1–4% NaCl, pH 5–7. DNA G+C content was 42.6 %. The result of 1500 b.p.16S rRNA gene sequencing analysis was 99.0% closely related with *S. parasanguinis*. *Og* had a distinctively independent branch of the phylogenic tree. DDHA with *S. parasanguinis* showed less than 70% similarity. Conclusions: Phenotypic and genotypic features are adequate to distinguish *Og* from other microorganisms. Molecular findings support that acid tolerant *Og* should be classified as a new microorganism rather than as a *Streptococcus* species. The bacterium is the first Gram-negative *Streptococcaceae*.

P267. Comparative genomics of UK and Israeli Campylobacter coli clinical isolates

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Introduction: *Campylobacter coli* is responsible for 10–20% of the cases of campylobacter-associated illness. Although consumption of undercooked poultry products is a risk factor shared with *C. jejuni*, there are unique risk factors for *C. coli*, which include swimming and consumption of game and tripe. Despite the importance of *C. coli*, this bacterium has received relatively little attention with regard to geographic differences, genome content and molecular biology. In this study we have analysed the genome sequence of 1 UK and 3 Israeli clinical isolates of *C. coli*. Methods: Genome sequencing of UK isolate Cc42yr and Israeli isolates Cc44856, Cc79361 and Cc81972 was carried out on the Illumina MiSeq. Preliminary annotations were generated using XBase with the genome sequence of *C. coli* RM2228 as comparison. Results: Each of the four genomes was successfully assembled into ~20 contigs, with a total genome size of approximately 1.7 Mbp. Although MLST-typing indicated that all four isolates are part of the ST-828 clonal complex, the Cc42yr and Cc81972 isolates clustered independently from the Cc44856 and Cc79361 isolates. Comparative genomics will aid characterisation of the full gene and plasmid content of these four isolates, and linkage with phenotypic analyses. Impact: Despite its importance as foodborne pathogen, relatively little is known about the specific molecular mechanisms underlying *C. coli* biology and virulence. Comparative genomics applications will inform future studies, but will require *C. coli* genome sequences like those presented here of recombination between these porcine *C. jejuni* and related Campylobacters

P268. Utility of Capsular Multiplex PCR as an Epidemiological Tool for Investigation of Clinical *Campylobacter jejuni* Isolated from Thailand.

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Intro: The subtyping of *Campylobacter jejuni* isolates is considered essential for epidemiological purposes. The polysaccharide capsule of *C. jejuni* is the major serodeterminant of the Penner serotyping scheme. A multiplex PCR method for determination of capsule types of *C. jejuni* which is simpler and more affordable than classical Penner typing was developed and validated.

The aim of this investigation was to compare the usefulness of capsular multiplex PCR, PFGE and MLST techniques for subtyping of *C. jejuni* strains, in terms of user-friendliness and discriminatory power. Methods: 322 *C. jejuni* strains were isolated from traveler's diarrhoea in Thailand. Isolates were genotyped with PFGE (*Sma*I/*Kpn*I), MLST and three capsular multiplex PCR sets using 25 different primers recognized 27 Penner's and 3 complexes serotypes. Results: Capsular multiplex PCR typing was less discriminatory than PFGE and MLST typing (DI = 0.911), and many strains possessed common types including HS23/36 complex (48/322), HS4 complexAB (47/322) and HS53 (45/322), whereas PFGE typing gave the highest discriminatory power (DI = 0.988). However, capsular multiplex PCR typing was simpler than other genotyping and this method was confirmed with 100% sensitivity and with specificity ranging from 96–100% using 103 known Penner type strains. Impact of research: Capsular multiplex PCR is a rapid and easy to use method with acceptable discriminatory power. The ability to rapidly determine capsule type may also help determine if specific *C. jejuni* capsule structures are associated with severe disease. However, this technique may be further improved by designing primers specific for remaining untypable strains.

P269. Genome analysis of *Campylobacter jejuni* strains isolated during a waterborne outbreak

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Introduction: Waterborne outbreaks associated with *Campylobacter jejuni* are rather common in the Nordic countries. PFGE is used to confirm the association between the isolates from water and patients. However, variability in PFGE patterns has been observed and bacteriophage translocation has been supposed to be responsible for this event. To evaluate the genomic plasticity of *C. jejuni* in a course of an outbreak, genome wide comparison of three outbreak-associated strains, one from water and two from patients, was performed. Methods: The genomes were sequenced using Illumina technology. Each genome was independently scaffolded using 5 kb mate-pair library. The genomes were assembled using MIRA and annotated using RAST annotation server and manually inspected. To identify changes in synteny, the genomes were aligned using MAUVE and BLASTN. Single Nucleotide Polymorphisms (SNPs) were identified by mapping the Illumina reads to the genome of the *C. jejuni* strain obtained from water. Results: A patient *C. jejuni* strain having the same PFGE profile of the water strain, showed identical synteny and two SNPs. The second patient strain, displaying a little variation in the PFGE pattern, showed differences, both in terms of gene content and nucleotide composition of a bacteriophage-like element similar to CJIE-2 of RM1221 strain. Impact of the research: Our data confirmed the role of bacteriophage in the genome plasticity of *C. jejuni*. We observed that not only the translocation but also the rearrangement of the gene content of the bacteriophage may be responsible of the variability of the PFGE patterns of epidemiologically associated strains.

P270. Assessment of DNA extraction methods and suitability of a PCR method for the detection and quantification of *Campylobacter* in broiler caeca samples

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Introduction: Accurate and rapid PCR methodology to detect, identify and quantify *Campylobacter* species in chicken caecal contents and farm samples could facilitate practical testing solutions that are applicable for monitoring and control measures. However, more information is needed regarding optimal DNA extraction methodologies and PCR test performance. This study assessed the sensitivity and specificity of a PCR test for *Campylobacter* detection. The potential for quantification and the influence of DNA extraction methods were also assessed. Methods: Pools of ten caecal samples from broiler flocks at slaughter were cultured for *Campylobacter* detection using the ISO-10272 method. Subsets of samples were also tested by enrichment and enumeration methods. DNA was extracted from all samples by a manual resin column extraction system and DNA from a subset of samples was extracted by an automated system. All DNA preparations were tested using the *map*A/*ceu*E based assay for the detection and differentiation of *C. jejuni* and *C. coli* respectively. Results: The sensitivity of the PCR method was 89.7% and 85.9% for the detection of *C. coli* and *C. jejuni*, respectively and specificity was 100%. The ct value obtained by PCR appeared to be related to the *Campylobacter* level in the sample and the lowest level of contamination detected was 10^6 cfu/g. PCR results obtained from extracts by the automated method were similar to manual extraction. Impact: PCR is useful to rapidly detect highly contaminated flocks and to provide highly specific species identification, providing useful insight into flock co-infection.

P271. Development of a novel mCCDA formulation to minimise the growth of competing ESBL producing bacteria during *Campylobacter* culture

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Introduction: In poultry-associated samples there are increasing numbers of reports of cephalosporin resistant bacteria as a result of extended spectrum beta-lactamase production. These bacteria, typically *E. coli* and cefoperazone resistant, can grow on mCCDA, interfering with detection and enumeration of *Campylobacter* (ISO-10272 methods). This study aimed to determine minimal inhibitory concentration (MIC) profiles for ESBL producing bacteria and *Campylobacter*. Using these MIC profiles a new formulation of mCCDA was developed and tested to minimise interference of competing bacteria when enumerating *Campylobacter*. Methods: A panel of 46 ESBL-producing *E. coli* from broiler and livestock sources and a representative selection of 40 *C. jejuni* and 13 *C. coli* from an UK national survey of broiler chickens were selected for MIC determinations. MIC analysis was by an iso-sensitest agar (ISA) plate dilution method for a range of antibiotic combinations including Beta lactamase inhibitors. Antibiotics that were inhibitory for *E. coli* but not for *Campylobacter* were then assessed in a mCCDA formulation for MIC. An optimal media formulation was then assessed for *Campylobacter* enumeration performance (productivity and selectivity) in carcase rinse samples. Results: *Campylobacter* were more resistant than *E. coli* to tazobactam/cefoperazone, colistin and polymyxin B in both ISA and mCCDA. An optimal mCCDA formulation with colistin was tested on neck skin samples and this demonstrated a 6 $\log^{10}$ reduction in *E. coli* counts with no reduction in *Campylobacter*. Impact: The use of mCCDA with colistin may minimise the risk of contamination/interference in ISO-10272 methods without lowering test sensitivity.

P272. Low Cost Semi-Continuous Quantification of *Campylobacter* by Air Sampling in Broiler Houses

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Campylobacter is one of the leading causes of acute diarrheal disease worldwide. It is therefore relevant to control and reduce levels of *Campylobacter* in broilers which are the main reservoir for human infection. This study describes an evaluation of a semi-continuous method for quantification of *Campylobacter* by air sampling in broiler houses. To identify control measures that would be universally applicable sampling was carried out in conventional broiler houses in Poland in addition to preliminary samplings in Denmark. Each measurement consisted of air samples on gelatin filters, conventional boot swab faecal samples and particle counts. Sampling was conducted over an 8-week period in three flocks assessing the presence and levels of *Campylobacter* in boot swabs and air samples using culture and quantitative real-time PCR (qPCR). The particle counts were used to analyse size distribution in airborne particles (0.3–10 μm) in the broiler houses in relation to bacterial distribution. No correlation between airborne *Campylobacter* and a specific particle size was found. *Campylobacter* was first detected in the flocks after 0 and 2 weeks using air sampling and boot swabs, respectively. All samples were positive for *Campylobacter* from week 2 and the rest of the rearing period with both methods, though 1–2 logs higher levels (*Campylobacter* cell equivalents/sample) were found with air sampling. In conclusion, air sampling on filters, coupled with qPCR, was able to detect *Campylobacter* colonization before it could be detected by boot swabs and found to be a promising technique for monitoring of *Campylobacter*.

P273. Whole-genome sequencing for elucidation of evolution and fitness of an emergent *Campylobacter jejuni* clone associated with sheep abortion in the USA

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Introduction: Outbreaks of *Campylobacter*-associated ovine abortion impose economic burden worldwide, and are tradinionally caused by multiple species and strains of this zoonotic organism. However, a highly pathogenic clone of *C. jejuni* (clone SA) has emerged as the predominant cause of recent sheep abortions in the USA (since 2003). This study aimed to ascertain the adaptive genetic changes responsible for the emergence and predominance of clone SA. Methods: Illumina sequencing was used to analyze whole-genomes of *C. jejuni* clone SA strains from sheep abortions since 1990s.

Using the previously sequenced complete genome of IA3902 (a clone SA strain) as scaffold, the newly sequenced genomes were assembled and comparative genomics were performed *in-silico* to reveal genetic changes in clone SA on a time scale. Results: As determined by mapping of sequence reads to the reference genome, the numbers of SNPs in each genome showed a positive correlation with the year of isolation of corresponding strains relative to that of IA3902 (i.e. 2006). SNP-based phylogenetic tree separated the genomes based on isolation year, placing the historical genomes (early 1990s) in a distinct cluster from those of epidemic isolates (post-2003). Comparative genomics of the *de novo* assembled genomes (consisting of 62–84 contigs) indicated an overall high degree of genomic synteny and sequence homology. Impact of research: These sequences revealed the genomic differences in clone SA isolates before and after its epidemic rise as the cause of sheep abortions, and thus provide insights into the genetic events underlying its emergence, evolutionary history and increased fitness.

P274. Cj0588 protein *Campylobacter jejuni* is the 23S rRNA methyltransferase

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Campylobacter jejuni Cj0588 protein is an ortholog of proteins named TlyA found in *Mycobacterium* sp. TlyA proteins modify 2′-hydroxyl groups of cytidine on ribosomal subunits. TlyA proteins were segregated into two groups: first $TlyA^{I}$ that methylates nucleotide C1920 in 23S rRNA and second grupe $TlyA^{II}$ that methylates C1409 in 16S rRNA in addition to nucleotide C1920. It correlates with lengths of N- and C-termini of $TlyA^{I}$ and $TlyA^{II}$ protein. Cj0588 belongs to $TlyA^{I}$, it does not have four N - terminal aminoacids (A2-R3-R4-A5) and C - terminus is about twenty aminoacids shorter. Three-dimensional modeling of Cj0588 showed a typical for RNA methyltransferase structure: seven-stranded β-sheets between five α-helix layers and three residues K^{80}-D^{162}-K^{188} corresponding to catalityc triad. Methyltransferase activity of *C. jejuni* Cj0588 protein was examined. The purified recombinant Cj0588 protein incorporates of radioactive methyl group of S-adenosyl-L-methionine (AdoMet) into the 23S rRNA in 50S ribosomal subunit *in vitro*. The enzymatic properties of wild type Cj0588 was characterized. The V_{max} of the methyltransfer reaction, as well as the K_m values for 50S ribosomal subunits and AdoMet, were determined by *in vitro* methylation assays using purified Cj0588 and radioactively labeled AdoMet. The K_m for AdoMet was 4.6 μM, K_m for 50S ribosomal subunits was 0.2 μM and K_{cat} 0.004 min^{-1}. To characterize active site of Cj0588 protein, we prepared site-specific mutants (in K^{80}-D^{162}-K^{188}) to investigate which residues participate in catalysis. L^{188} aminoacid appear to play significant role in the methyltransferase reaction *in vitro*.

P275. Characterization of *Campylobacter jejuni* biofilm formation using confocal laser scanning microscopy

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Campylobacter jejuni is nowadays one of the most common causative agents of gastrointestinal diseases. Although this pathogen is highly sensitive to external conditions, it is able to survive in food processing conditions and is responsible for cross- or re contamination of food products. One of the survival strategies might be its ability to adhere to abiotic surfaces and form biofilm. The aim of this work was to decipher the biofilm structure and development using laser scanning confocal microscopy. Strains of *C. jejuni* were cultivated up to 3 days in Brain Heart Infusion at 37°C in microaerobic or oxygen enriched atmospheres. Samples were then stained with systemic and specific fluorescent probes. We observed that *C. jejuni* is able to form mushroom-like biofilm structure within 24 hours of cultivation. Original data obtained about cell motility, biofilm matrix, cell distribution in the biofilm and effect of oxygen-enriched conditions will be presented.

P276. Comparative genomics of *Campylobacter fetus* subspecies

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Campylobacter fetus subspecies infect hosts and niches with distinct preferences. *C.fetus fetus* (Cff) is isolated from a variety of sites in different hosts whereas *C.fetus venerealis* (Cfv) is restricted to the genital tract of both male and female cattle. Cfv includes a variant, designated Cfv biovar intermedius (Cfvi). To understand the different host preferences a whole genome (WGS) comparison of the *C. fetus* subspecies was performed. Roche 454 FLX sequencing was performed for 21 *C. fetus* strains (4 Cff and 17 Cfv). For Cff strain 04/554, Cfv strain 97/608 and Cfvi strain (01/165) Illumina MiSeq data were added. Using Perl scripts the contigs of Cff 04/554 and Cfv 97/608 were assembled into single scaffolds that were confirmed with optical restriction maps (OpGen). Scaffold genomes were annotated against the genome annotation for Cff strain 82-40. The gene content was determined with similarity searches using a local BLASTN tool and other epsilonproteobacterial genomes. Cff contained a 26 kb plasmid, Cfv carried a 27 and 38 kb plasmid. All optical maps were collinear with the Cff 82-40 map. Cfv and Cfvi contain multiple insertion sequences (IS) and integrated phage sequences. Both subspecies contain a variable S-layer region and CRISPR-Cas locus. A strong conservation of >99% average amino acid identity between the *C. fetus* core proteomes was observed. Comparison of gene composition is in progress. The *C.fetus* subspecies genomes are highly syntenic. The presence of multiple gene-inactivating IS elements and integrated phage/islands in Cfv and Cfvi may contribute to the Cfv biology.

P277. The *Campylobacter jejuni* CRISPR-Cas system represents a specific subtype of Type IIa CRISPR-Cas phage defence systems

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Introduction: The Clustered Regularly Interspaced Short Palindromic Repeats and associated genes (CRISPR-Cas) system defends the genome of bacteria and archeae against bacteriophages and other forms of foreign DNA, with the mature CRISPR RNAs (cRNAs) molecules targetting the incoming DNA for degradation. Many *Campylobacter jejuni* strains have a Type IIa (Nmeni) CRISPR-Cas system, and in this study we have investigated the transcription, function and evolution of this subtype of CRISPR-Cas systems. Results: Type IIa CRISPR-Cas have only been characterised in *Streptococcus* species, where the mature crRNAs are generated by the Cas9 protein and RNase III from a single precursor RNA produced downstream of the Cas genes. In contrast, the *C. jejuni* CRISPR array was transcribed at very high levels, from multiple σ^{70} promoters located at the 3' end of each individual CRISPR repeat which convergently oriented to the Cas-genes, rather than downstream. BLAST searches identified CRISPR spacers matching genomes of *Campylobacter*-specific phages, supporting a role in phage defence. Comparative genomics of Type IIa CRISPR-Cas systems suggested that *C. jejuni* CRISPR-Cas represents a subtype of the Type IIa systems, separate from the streptococcal one, and has a short Cas9 protein (~120 kDa) and only three Cas genes, rather than a large Cas9 protein (~160 kDa) and four Cas genes. Phylogenetic analyses showed that CRISPR repeat sequence and subtype of CRISPR-Cas were linked, suggesting these are transferred jointly by horizontal gene transfer. Impact: This study highlights that even within the Type IIa CRISPR-Cas system, variability exists in transcriptional patterns and Cas genes.

P278. Comparative study of different sampling methods for enumeration of *Campylobacter* from broiler carcases

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Introduction: The performance of sampling and testing a pool of three neck skin samples per batch for enumeration of *Campylobacter* (Pool method (PM)) was compared with sampling and testing neck skin from one single carcase per batch (EU method (EUM)). The assessment of comparative performance of the two sampling methods included estimates of (i) the within-batch prevalence (ii) the variability of *Campylobacter* counts (cfu/g) in contaminated carcasses and (iii) the impact of pooling. Caecal counts and the effect of the initial dilution used for the carcase were also assessed. Methods: The comparative study was conducted in 17 slaughter batches; for each batch, 10 samples were tested by the EUM and another 10 samples were tested by the PM. *Campylobacter* levels in 10 caecal samples per batch were also determined. Two different ratios for the initial suspension (1:2 vs. 1:9) were compared for neck skin samples from 6 slaughter batches. Samples were enumerated by direct plating on mCCDA following ISO 10272: Part 2. Results: Within a batch, the percentage of samples with a contamination level above 3 log10 cfu/g varied between batches, but overall it was higher by the PM than by the EUM. For batches with high variability of counts amongst individual samples, the pooled sample resulted in lower *Campylobacter* counts. Impact: The within and between batch variability of *Campylobacter* counts in carcase samples and the impact of different sampling and testing strategies should be taken into account for accurate estimation of the *Campylobacter* contamination level in broiler batches.

P279. The role of lipid asymmetry in maintaining the integrity of the outer membrane of *Campylobacter jejuni*

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The outer membrane (OM) of *C. jejuni* is a key factor in the interaction with the host; it acts in adhesion, interaction with the immune system, cell signalling and as a nutrient barrier. The OM has an asymmetric distribution of lipid, with the outer leaflet containing lipid A conjugated to oligosaccharide (LOS) and an inner leaflet containing phospholipids (PLs). Under stress conditions, PLs are forced to move from the inner leaflet and accumulate in the outer leaflet of the OM. To maintain lipid asymmetry, PLs have to be destroyed or moved to the cytoplasmic membrane. In *E. coli*, a new ABC (ATP-binding cassette) transport system has been identified that maintains lipid asymmetry in the OM, named Mla (maintenance of lipid asymmetry). In *C. jejuni*, homology searches lead to the identification of three *mla*-like genes; *cj1371* (*mlaA*), *cj1372* (*mlaC*) and *cj1373* (*mlaD*). We investigated the role of these genes in the maintenance of the OM by growth, sensitivity and motility assays, and biofilm formation, in *mla* mutants. Interestingly, we found that under stress conditions single deletion of *mlaA* and *mlaC* resulted in a severe growth defect, increased sensitivity to antimicrobial agents, lowered biofilm formation and increased spreading motility. We suggest that these phenotypes reflect the essential role of the Mla transporter in maintaining lipid asymmetry in the OM of *C. jejuni*, where mutation results in membrane instability. Our results provide new insight into the importance of OM integrity in *C. jejuni* and identify a new target for antimicrobial intervention.

Other

P280. Campylobacteriosis in New Zealand*:* A new twist to the tale?

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New Zealand has a much higher rate of reported campylobacteriosis cases than the rest of the developed world. It has previously been assumed that either strains of New Zealand *C. jejuni* have greater heat tolerance or that they are more oxygen tolerant. However, an alternative hypothesis suggested is: whether secondary processing practices may increase the contamination level of chicken and whether New Zealanders have worse home hygiene practices during food preparation than the citizens of other developed countries. The investigation revealed that New Zealand strains are not more heat resistant or oxygen tolerant than other strains. The results from the secondary processing experiments revealed that secondary practices investigated at a plant did not significantly increase the contamination level of the carcasses. A QMRA study using the Bayesian approach has indicated that hygiene has a significant impact on the total probability of illness. The findings of the QMRA and a review of consumer food handling practice confirm the hypothesis that poor hygiene may contribute to the high rate of campylobacteriosis. A strategy announced in 2006 expected a 50% reduction in the rate of notified campylobacteriosis cases over the next five year period. However, unexpectedly the reduction occurred in 2007 and 2008. This reduction appears questionable as it occurred in a very short time, and the poultry data of two poultry plants did not show a remarkable decrease in the level of contamination or prevelance. This negates the claims that the decline is attributable to the interventions implemented in the poultry industry.

P281. The effect of Maillard reaction products formed from honey in the growth, morphology and virulence of *Campylobacter jejuni*

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Campylobacter is becoming the major cause of bacterial enteritis in both developed and developing countries. The main sources of infection are undercooked meats and milk. The addition of natural products such as honey to these foods may help in reducing campylobacter contamination. However, natural products such as honey may go through different reactions during thermal treatment and other food processes. Interestingly, we have shown that heating honey with casein develops antibacterial agents which are active against *Campylobacter jejuni*. The antibacterial mixture was prepared by mixing honey and casein in an equal amounts, dissolving in sterile distilled water then heating at 121 °C for 15 minutes. The mixture was then added to Muller Hinton media at different concentrations and then tested against *Campylobacter jejuni* NCTC 11168 to determine the minimum inhibitory concentration (MIC). Further antibacterial activity studies of the MIC (2% v/v) were investigated using growth curves, survival in liquid media as well as milk. In addition, the mixture showed a significant effect on the ability of *Campylobacter jejuni* to invade mammalian cells when tested using a Caco-2 cell line (2 log reduction). Transmission electron microscopy pictures confirmed the change in *Campylobacter jejuni* morphology after different periods of treatment. This study shows the presence of important active antibacterial compounds in this honey-casein mixture. Identifying them and potentially using them may help in reducing hazards due to the presence of *Campylobacter jejuni* in food.

P282. Prevalence of Thermotolerant *Campylobacter* Strains Isolated from Poultry Carcasses in The Region of Tlemcen, Algeria

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There is a glaring lack of data on food poisoning caused by *campylobacter* in Algeria; we have decided to conduct this study which aims to determine the presence of thermotolerants *Campylobacter* in poultry carcasses. The research was carried out

between May 2012 until March 2013 in 2 poultry slaughterhouses in region of Tlemcen, followed by an examination of poultry on the market. A total of 210 samples were collected including carcass surface swabs, rinsing water and chicken meat (necks, thighs and wings). The methods used to recover *Campylobacter* from each sample type were (i) direct plating on CFA (campy food agar), (ii) direct plating on mCCDA, (iii) Preston broth enrichment and subsequent inoculation onto CFA, (iv) Preston broth enrichment and subsequent inoculation onto mCCDA, and (v) Preston broth enrichment and subsequent inoculation onto Karmali medium. Incubation at 42+ 0.5°C for 48 h under microaerophilic conditions (5% O2, 10% CO2 and 85% N). We found a very high and concerning prevalence of thermotolerant *Campylobacter* about 93%. The identification tests were performed by the Api*Campy* plates. Of the 226 Campylobacter isolates, 83.33% were identified as *Campylobacter jejuni* (27.77% *C. jejuni ssp doylei*, 47.22% *C. jejuni ssp jejuni1*, 5.55% *C.ssp jejuni2* and 2.77 *C.ssp jejuni3*), 11.11% isolates were identified as *C. coli* and 5.55% as *C. upsaliensis*. The study concluded that very high proportion of chicken meat and chicken by-products marketed in Tlemcen area are contaminated by Campylobacter, with a possible risk from such microorganism especially from consumption of undercooked or post-cooking contaminated chicken products.

P283. Pre-clinical evaluation of KatA as a vaccine candidate against *Campylobacter jejuni* in mice

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Campylobacter jejuni (*C. jejuni*) is implicated as one of the main biological agent in food poisoning cases around the globe. Commonly found in animal feces, *C. jejuni* infection results in severe enteritis characterized by diarrhoea, abdominal pain and muscle pain. Due to lack of commercial vaccines, there is a need to develop safe and effective vaccines against *C. jejuni* infection. Towards this, we constructed a protein subunit vaccine using catalase -KatA protein from *C. jejuni* as our vaccine antigen. KatA protein vaccine was administered with alum and or monophosphoryl lipid A (MPL) adjuvants at weeks 0, 3 and 6 in cohorts of female BALB/c mice via intramuscular inoculations and sera was collected two weeks post-last vaccination. Mice that received KatA without adjuvants had anti-KatA IgG end point titers of 1.9×10^6 with at Th2 bias. Comparable mean ELISA titers (3.9×10^6 and 3.8×10^6) were observed in mice immunized with KatA in conjunction with alum and MPL respectively. Mice that received formalin inactivated *C. jejuni* had a Th2 bias with mean end point ELISA titers of 2.5×10^6. Mice immunized with KatA in conjunction with both adjuvants (alum+MPL) had the highest titers (5.6×10^6) among all vaccinated mice with a mixed Th1 and Th2 response.We are currently performing experiments to evaluate the bacteria-neutralizing ability of the sera harvested from different vaccine candidates. This will help us to move forward with the best vaccination approach and develop an effective disease prevention strategy against food poisoning.

P284. High prevalence of thermotolerant *Campylobacter* ssp in Swedish raw waters

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Although food is the predominant reason for human campylobacteriosis, drinking water is not negligible as a source of infection. In Sweden, *Campylobacter* ssp have been linked to approximately 20 drinking water borne outbreaks since 1980 of which several have rendered thousands of consumers ill. This makes *Campylobacter* the most common cause of drinking water borne outbreaks, and also illustrates the potency of drinking water for disease transmission. Here, we examined the prevalence of thermotolerant *Campylobacter* in raw waters across Sweden with two week intervals during a full year. Species identification of isolates by PCR methods showed an almost even distribution between *C. jejuni*, *C. coli* and *C. lari*. Although no geographical differences could be seen, there was a clear seasonal variation with highest prevalence of positive samples in September-January while only few samples were positive from February until mid-June. This variation correlates well with other water quality parameters such as coliform bacteria and E. coli. Together, the results indicate different sources of risk in raw waters and also demonstrate when drinking water producers should be particularly cautious of contamination with *Campylobacter*. The present survey is part of a larger study intending to clarify the significance of different sources of human campylobacteriosis. In this source attribution study, isolates from raw water, wild birds, cattle, pigs, sheep, dogs and chicken are analysed, using MLST as genotyping tool. Also, we have initiated an extended survey of raw waters that includes other drinking water related pathogens such as *Salmonella*, VTEC, *Cryptosporidium*, *Giardia* and Norovirus.

P285. Collection of *Helicobacter Pylori* Clinical Isolates to Facilitate The Development of A Novel Vaccine

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Helicobacter pylori infects more than 50% of the world's population, resulting in peptic ulcers and gastric carcinomas. Infection rates are disproportionately high among First Nations people in Canada where factors such as crowded living conditions and limited access to running water may hinder the ability to control these infections. Currently, there are no vaccines against *H. pylori* nor is there any epidemiological data concerning this bacteria for Northern Ontario. As such, our goals are to establish a collection of *H. pylori* clinical samples from residents of Northern Ontario, and to use the data collected to facilitate the generation of a novel vaccine. The aims of this project are 1) to enroll hospital patients who are normally scheduled for gastroscopy as part of their care, 2) to build a collection of *H. pylori* isolates through positive identification by biochemical testing and PCR, and 3) to validate applicability of a vaccine candidate to local phenotypic and genotypic variations. Currently, while *H. pylori* is being identified by positive urease, catalase, and oxidase reactions, PCR using *ureC* primers has been the most consistent indicator of the presence of bacteria. This study is important to learn about patterns of *H. pylori* distribution, and will aid in the design of the best vaccine candidate to protect susceptible populations. In addition, since epidemiology and culturing are not well established in Northern Ontario, this study provides an excellent opportunity to expand clinical programs and fulfill the need for a solid disease surveillance program in the region.

P286. Antibiotic resistance and genetic diversity of Human, Food and Animal origin Campylobacter spp. isolates from Portugal

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Infections by *Campylobacter jejuni* and *C. coli* are considered the major cause of bacterial gastroenteritis in humans, being the consumption or handling of contaminated food the major source of infection. The use of antibiotics in food animals' production and veterinary treatments is contributing to the increasing of antibiotic-resistant *Campylobacter* in food products of animal origin, and consequently the raise of multiresistant human isolates. A total of 196 *Campylobacter* strains, 125 isolates from humans (78 *C. jejuni*; 47 *C. coli*), 39 (6 C. *jejuni*; 33 *C. coli*) from retail food, and 32 (4 *C. jejuni*; 28 *C. coli*) from animal sources samples, were studied for the susceptibility to seven antibiotics, by the agar dilution method, as well as for the molecular determinants of antibiotic resistance. Strains were genotyped by MLST and *flaA*-SVR sub-typing. Overall, a high antibiotic-resistance rate to five antibiotics was observed, with ciprofloxacin-resistance rate exceeding 90%; resistance to macrolides and aminoglycosides were 25% and 1.6%, respectively, with emphasis on the 56% macrolide-resistance among animal source isolates. Besides the expected point mutations associated with antibiotic resistance, the detected polymorphisms in the *cmeABC* locus likely play a role on multi-resistance phenotype. Genotyping showed that within each species, genetically related isolates are present in different sources. *C. coli* strains were genetically more conserved, with a predominant clonal complex (CC-828), in contrast to *C. jejuni*. We highlight a worrying antibiotic multi-resistance rate among *Campylobacter* isolates from Portugal and the emergence of strains resistant to antibiotics of human use.

P287. Inhibitory effect of resveratrol against *Arcobacter butzleri* and *Arcobacter cryaerophilus*

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Some species belonging to the genus *Arcobacter*, family *Campylobacteraceae*, are emerging pathogens that have been linked to human enteritis and more rarely bacteraemia. Its detection in food products, associated with the increased antimicrobial resistance of foodborne pathogens to common food preservatives led to the demand for new strategies to control these pathogens. Resveratrol is a plant derived chemical with several health benefits, besides exhibiting promising antimicrobial activity against a number of bacteria and fungi. The activity of resveratrol against *Arcobacter cryaerophilus* and *Arcobacter butzleri* was assessed by microdilution method, and the minimum inhibitory concentration was 50 and 100μg/mL, respectively. Killing curves revealed that the inhibitory effect was dependent on growth phase. Furthermore, the effect of resveratrol on cellular functions was evaluated by flow cytometry using deep red-fluorescing bisalkylaminoanthraquinone number five (DRAQ5) for the analysis of intracellular DNA content and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) for the evaluation of metabolic activity. The incubation with resveratrol led to a decrease in both intracellular DNA content and respiratory activity with no significant change in CTC-reducing cells with higher resveratrol concentrations. Overall, these results suggest that resveratrol may affect DNA synthesis in both *Arcobacter* species, leading to an increase of cells with lower DNA content, which can result in an impairment of cell division. Concomitantly, the higher cellular stress caused by resveratrol resulted in a marked decrease in *Arcobacter* metabolic activity. We conclude that resveratrol can be potentially exploited by the food industry as a natural solution for the prevention of *Arcobacter* growth in food.

P288. Spatial and temporal colonization dynamics of segmented filamentous bacteria is influenced by gender, age and experimental infection with *Helicobacter hepaticus* in Swiss Webster mice

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Uncultivable segmented filamentous bacteria (SFB) are part of commensal microbiota present in a wide spectrum of animal species and are recognized as a potent inducer of proinflammatory TH17 cells, thereby playing an important role in host immunity. In this study, we established a 16S rDNA-based qPCR assay for quantifying SFB with high sensitivity and specificity, which was then utilized to determine colonization levels of SFB in jejunum, ileum, cecum and colon of Swiss Webster (SW) mice at both 8 and 16 weeks post-inoculation (wpi) with *H. hepaticus* 3B1 (Ge *et al.*, IAI 73:3559, 2005). At 8 wpi, SFB were predominantly present in the jejunum and ileum of sham controls; cecal and colonic SFB positivity was significantly increased at 16 wpi compared to that at 8 wpi. At 8 wpi, *H. hepaticus* infection did not alter SFB colonization levels in jejunum and ileum of both mouse genders, but was associated with higher colonization levels of SFB in the cecum and colon of males compared to controls. At 16 wpi in males, there was no significant difference in SFB levels in in the jejunum, ileum, cecum and colon between 3B1-infected and control groups, whereas the 3B1-infected females contained lower levels of SFB in the jejunum, cecum and colon compared to controls. These results demonstrate that there was increased colonization of SFB in the large intestine during aging of SW mice and SFB colonization dynamics were altered by *H. hepaticus* infection in a gender-dependent manner.

P289. Distribution of Arcobacter spp. in two poultry processing plants in Thailand

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Introduction: *Arcobacter* is an emerging foodborne pathogen. Although *Arcobacter* has been detected in foods of animal, higher prevalence of this organism has been found in chicken carcasses. Recently, the route of *Arcobacter* infection and the origin of carcass contamination are still unknown. The purpose of this study was to determine the distribution of *Arcobacter*

spp. and the possible route of carcass contamination in Thai poultry processing plants. Materials and Methods: In total, 504 samples of cloacal swabs, slaughterhouse environments and chicken carcass rinse were collected from two poultry processing plants. *Arcobacter* was isolated using the membrane filtration technique after enrichment in *Arcobacter* enrichment broth (AEB). All isolates were confirmed and identified to species level by multiplex PCR assay and further characterized by repititive sequence-based PCR (rep-PCR). Results: Our results show that two poultry processing plants were moderately contaminated with *Arcobacter*, which *A. butzleri* was the most common species. The frequency of *Arcobacter* spp. ranged from 28.6–71.4% before defeathering and reached almost 100% after defeathering. After evisceration, the contamination dropped to less than 70% and then increased to nearly 100% after chilling. Although only 15% of cloacal swabs from chickens were positive for *Arcobacter*, the recovery rates of this organism from finished chicken products were very high ranging from 75.0–93.8%. Discussion: Our results indicate that the contamination of *Arcobacter* in poultry carcasses likely occurs during slaughtering. Therefore, *Arcobacter* control strategies should focus on reducing contamination at the slaughterhouse level.

P290. EU Reference Laboratory- *Campylobacter* Proficiency Tests for National Reference Laboratories. Progress of performance in three years.

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The European Union Reference Laboratory (EURL) for *Campylobacter* organizes annual proficiency tests (PTs) for the National Reference Laboratories (NRLs) in EU as part of the tasks and duties of the EURL. In 2010 to 2012 the PTs included detection and enumeration of *Campylobacter* spp. in meat matrices, essentially by using the standardized protocols of ISO 10272. The number of participating NRLs were about the same, 2010 (n=34), 2011 (n=34), 2012 (n=36). The number of NRLs that reported correct results of detection of *Campylobacter* in all samples after enrichment in Bolton broth followed by culture on mCCDA was 56%, 71%, and 47% in 2010, 2011, and 2012, respectively. Corresponding figures for enrichment in Bolton broth followed by culture on a second selective agar were: 64%, 70% and 62%. After enrichment in Preston broth followed by culture on mCCDA, 66% 71% and 69% of NRLs reported correct detection of *Campylobacter* in the years 2010–2012. The samples with most incorrect results were samples which contained a mixed culture of *Campylobacter* and *E. coli*. For assessing the results, a five grade scoring system was used. The overall performance in enumeration of *Campylobacter* spp. has improved during the years; 64% (2010), 79% (2011) and 85% (2012) of the NRLs received the grade "excellent performance". In 2012, the majority of the NRLs performed excellent or good in all parts, detection, species identification, and enumeration meeting the requirements of being an NRL. Assistance from the EURL is provided to NRLs that need to improve their performance.

P291. Source attribution of sporadic *Campylobacter jejuni* infections in the United States

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Microbial subtype-based source attribution models have been successfully used for identifying intervention targets to reduce *Campylobacter* infections. Here we present preliminary source attribution modeling data for *C. jejuni* infections in the United States based on microbial subtyping. Sporadic *C. jejuni* case isolates (n=789) from 1998 and 2008 submitted by the 10 Foodborne Diseases Active Surveillance Network sites were characterized by multi-locus sequence typing (MLST) and attributed to source isolates (n=8372) described in 65 published studies, using a Dutch and an adapted Danish model. A subset of source isolates from the U.S. was also analyzed using the Dutch model. The Dutch and the adapted Danish models attributed most *C. jejuni* isolates to poultry (40% and 53%), followed by cattle (19% and 17%), sheep (14% and 9.4%), the environment (14% and 13%), wild birds (7.2% and 5.9%) and pigs (5.0% and 1.8%), respectively. Using only U.S. source data, the Dutch model attributed the sources of most isolates to poultry (37%), followed by cattle (21%), sheep (15%), the environment (13%), wild bird (5.2%) and pigs (2.9%). We present preliminary *Campylobacter* source attribution data for the U.S. based on microbial subtyping. Using international source data, the Dutch and Danish models provided highly congruent attribution estimates for each source. Poultry was identified as the primary source of infection in our dataset, followed by ruminants and the environment. Additional U.S. source data is needed to improve the current domestic model. Work in progress using the asymmetric island model will also be presented.

P292. Western Blot Assay with the 36.1kDa Cell Wall Protein of *C.jejuni*

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Contaminated poltry meat is the major vehicle for human campylobacteriosis. The use of antibiotics in flock could›t be a rational solution to prevent *Campylobacter*iosis. The immunization can be alternative the use of antibiotics. Studies with broiler has identified IgY antibodies confer protection against *Campylobacter* colonization on young chickens. Here, using formalin-fixed C.jejuni whole cell antigens (wca) via injection in hens the IgY response to 36.1 kDa cell membrane protein of C.jejuni have investigated the ability to create in eggs. WCA from eight strains of four predominant smaI-PFGE types were obtained as described by Draviyam. Broiler were immunized with the antigen mixture. This 1ml preparation injected into both upper thighs of each hens on the initial day of immunization. Subsequently, 1ml booster injections of this antigens were in the same manner, two times at 14 days intervals. The eggs from immunized hens were collected 7 and 2 days before immunization and 6 to 8 weeks after the completion of hen immunization.IgY extraction from eggs was done with Polson's precipitation. IgY were identified by IgY ELISA kit. Anti-*C.jejuni*-IgY was detected by WB. WB strips have prepared from PVDF membrane move on three blots. One of them is the 36.4 kDa C.jejuni membran protein as diagnostic marker, other bands were consisted IgY antibody and human serum globulin. The eggs of 12 of the 17 chickens were found positive collected on 8 weeks after the completion of immunisation. As aresult immunization of chickens with C.jejuni antigens can lead to the formation of protective antibodies.

P293. Incidence and Molecular Epidemiologic Characteristics of C.JejunI in Broller farms

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Here, we aimed to detect C.jejuni incidence in broiler, the effect of age, clonal relationships of strains with PFGE and antigens on the cell membrane of the dominant strains. A total of 3000 solid samples like breast, crops and intestinal tissue of each chicken were collected from 50 different commercial flocks. The samples designed four groups I, III, IV weeks old chicks and slaughtered chickens. Using a sterile forceps, three samples from each chick were taken under aseptic conditions. There then put into 250ml of Bolton broth with 5% horse blood. After incubation under appropriate conditions, samples were passaged to mCCDA mediums. Grown colonies were identified with morphology, hippurate hydrolysis, oxidase activities, cysteine and nitrate-reduction tests. From colonies identified as campylobacter then were identified by PCR-RFLP using the THERM-I and IV primers and AluI enzyme. The strains were confirmed as C.jejuni were evaluated in terms of clonal relationship with PulseNet one day PFGE protocol. Analysis of C.jejuni cell wall proteins was performed by method of Pechere-Melo used SDS-PAGE. C.jejuni was isolated in III, IV weeks old chicks and slaughtered chickens respectively 0.8%(2/250), %13.2(33/250) and %22(55/250). But I weeks old chicks couldn't be isolated.Isolates were distributed within the 32 cluster, and the largest cluster is 28 members, with SmaI-PFGE.The largest four cluster isolates was comprised 68.8%(62/90) of all isolates. As a result of SDS-PAGE selected 12 member from these 4 clusters; all isolates were detected 14.4, 25, 36.1, 45, 66.2 and 116kDa proteins presence. C.jejuni is shown in Broiler in fewer incidences. Certain genotypes were dominant among flocks. CADF 36.1 kDa located on C.jejuni cell walls 's major protein can be used as a diagnostic marker.

P294. Variations in Survival of *Campylobacter jejuni* Strains in Water

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Introduction: *Campylobacter jejuni* is the main bacterial cause of foodborne human intestinal disease. Infection in humans is frequently associated with consumption of undercooked poultry. However, the environment contributes to pathogen transmission and the ability to survive in water is crucial to this process. Aims: The aim of this study was to compare the survival of a diverse panel of *C. jejuni* strains in sterilized water at different temperatures. Methods: We tested the ability of a panel *C. jejuni* strains to maintain culturability on Blood Agar at a starting density of (8×10^5 – 4×10^6) CFU/ml in 100 ml

volumes of sterile distilled water at 4°C and 25°C by sampling at days 0, 1, 2, 3 and 10 (for 4°C), and 0, 1, 2, and 3 (for 25°C). The strain panel included representatives of widely studied strains (NCTC11168, M1), different clonal complexes (ST21, ST45, ST61) and isolates from different sources (human, chicken cattle, water, soil, wild birds and bank vole). Results: We identified differences between the strains with respect to the maintenance of colony forming ability at day 3 (for 4°C) and at day 1 (for 25°C). For example, whereas 66.2% of strain 1107 cells were still culturable after 3 days, for strain NCTC11168 only 21.7% remained culturable. Impact of research: Survival in water varies between different strains of *C. jejuni* with potential implications for strain transmission. Tests for cell viability and the expression of genes during survival in water can be used to elucidate the mechanisms underlying these variations.

P295. A comparative exposure assessment of *Campylobacter* infections in Ontario, Canada: identifying the data gaps and ranking relative risks.

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Human campylobacteriosis is the leading bacterial gastrointestinal illness and one of the most costly in Ontario, Canada. Its epidemiology is highly complex and still elusive involving various reservoirs and transmission routes. *Campylobacter* are mostly transmitted by food in Canada, with live poultry and poultry products being the main reservoir and transmission route, respectively. However, there is a growing body of evidence that other reservoirs and transmission routes (foodborne - other than poultry, waterborne, and environmental) are important in this disease. To better understand and rank risks of exposure, C-EnterNet, the Canadian integrated enteric disease surveillance system, adopted a source attribution method (a comparative exposure assessment) to rank relative risks of exposure for this disease. This comparative exposure assessment was performed with Ontario-specific data, to identify existing data gaps and areas for future research. Raw data collection occurred over two summers (2011 and 2012) in Ontario, Canada, to gather information on *Campylobacter* prevalence at recreational beach sites, on fruits and vegetables, and fish and seafood products. In addition, data were gathered in the C-EnterNet sentinel site on *Campylobacter* prevalence on retail meats and farms. Systematic reviews and meta-analysis were performed to capture prevalence and concentration information for *Campylobacter* in pets and petting zoos. Relative risks will be ranked and summarized, while incorporating a quality of evidence score to prioritize future research studies and key knowledge gaps.

P296. Revised Estimates of the Burden of Food-borne Illness in Canada

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Aims: Food-borne illness estimates help set food safety priorities and create public health policies. The Public Health Agency of Canada recently completed revised estimates of food-borne illness for Canada. There were two overall objectives: (1) calculate a more accurate estimate of domestically, acquired food-borne illness in Canada using current data and more robust methods and (2) identify knowledge gaps for further research. Methods: Estimates for 30 known pathogens and unspecified agents using data from Canadian surveillance systems (for years 2000–2010), relevant international literature and the 2006 Canadian census population were calculated. The analysis accounted for under-ascertainment as public health surveillance systems are subject to under-reporting and under-diagnosis. Estimates on the proportion food-borne and the proportion travel-related were incorporated for each pathogen. Monte Carlo simulations were performed to account for uncertainty. Major Findings: There are an estimated 4.0 million episodes of domestically acquired, food-borne illness each year in Canada (1.6 million episodes from 30 known pathogens and 2.4 million episodes from unspecified agents). The top four pathogens are (1) norovirus, (2) *Clostridium perfringens*, (3) *Campylobacter* spp. and (4) non-typhoidal *Salmonella* spp. Main Conclusions:

The revised estimates are more accurate and lower than the 2008 estimate because they use current data and more rigorous methods. Impact: Policy makers, industry, academia and other organizations can use the revised estimates to better inform policy, research, food safety risk assessments, education campaigns and other prevention and control activities - ultimately improving the health of Canadians.

P297. Polymorphisms in inflammation-related genes may be useful as biomarkers for detection of patients at risk for gastric cáncer.

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There is a need for biomarkers useful to identify patients at risk to develop gastric cancer (GC). Polymorphisms in inflammation-related genes may be associated with the development of *H. pylori*-associated GC. HLA class II molecules have a relevant role in the inflammatory response elicited by most infections. Natural Killer (NK) cells have an important role in inflammatory and innate immune responses. Aims. We aimed to analyze polymorphisms in HLA-DQ genes and in the receptors of NK cells, NKG2D (polymorphisms) and KIR (genotyping) as risk factors for GC in a Mexican population. Methods. We studied over 400 patients with either non-atrophic gastritis or GC patients, and asymptomatic individuals. Polymorphisms in DQA and DQB genes were studied using reference strand-mediated conformation analysis and sequence-specific primer techniques. The KIR genes (14 genes and two pseudogenes) were studied by PCR-SSP using a commercial kit (PEL FREEZ™). NKG2D SNPs were genotyped with the TaqMan Allelic discrimination method using the StepOneTM RT-PCR system. Major findings. Patients with DQA1*04:01 or DQB1*05:01:01 alleles were at risk for GC (OR 2.49 and 2.33, respectively). For NKG2D, the C allele and CC genotype of rs1049174 were associated with protection for GC (OR 0.59 and OR 0.49, respectively). For KIR, the inhibitory genotype telomere A1/A1 and A1/Bx were associated with protection (OR 0.35 and 0.23, respectively), and the activator genotype telomere B1/B1 with risk (OR 9.8) for GC. Main conclusion. We identified polymorphisms in inflammation-related genes that may be useful as biomarkers to identify patients at risk of GC.

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